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THE SYNTHESIS OF OPSOPYRROLE-DICARBOXYLIC ACID[1]

BY D. M. MACDONALD[2] AND S. F. MACDONALD

ABSTRACT

The methyl groups of the pyrroles I*a* and II*a* react with sulphuryl chloride to give dichloromethyl derivatives which hydrolyze to aldehydes. The pyrrole I*a* also forms a trichloromethyl derivative which hydrolyzes to the corresponding acid. Alkaline decarboxylation of the latter gives opsopyrrole-dicarboxylic acid, IV*a*.

To obtain further pyrroles related to the uroporphyrins, we have studied the halogenation of the methyl groups of I*a* and II*a*, and further transformations analogous to those which had been carried out on an isomeric pyrrole (6, 9). The bromination of both I*a* (13) and II*a* (unpublished) proceeds normally giving the α-bromomethyl pyrroles.

With two moles of sulphuryl chloride, both I*a* and II*a* presumably gave dichloromethyl derivatives, which were not purified but hydrolyzed directly to the corresponding aldehydes I*b* and II*b*. The former has also been obtained from I*a* with lead tetraacetate (16). The latter, II*b*, was hydrolyzed to V*a* which was converted to the oxime V*b*.

With three moles of sulphuryl chloride, I*a* presumably gave a trichloromethyl derivative, which hydrolyzed to I*c*. The pyrrole I*d* was obtained from I*c* by thermal decarboxylation, and hydrolysis then gave III, one of the pyrroles which have been postulated as intermediates in the biosynthesis of hemin (12). Opsopyrrole-dicarboxylic acid IV*a*, one of the hypothetical reduction products of the uroporphyrins, was obtained from I*c* by alkaline decarboxylation. The method was not quite parallel to the analogous synthesis of opsopyrrole-carboxylic acid (7), for the greater solubility of the product here necessitated the release of the free acid by ion-exchange rather than by acidification. The dimethyl ester IV*b* was an oil which did not give a crystalline picrate. The structure of IV*a* was confirmed by its degradation to opsopyrrole-carboxylic acid (3-methylpyrrole-4-propionic acid). Further, although the usual methods for converting opsopyrroles to porphyrins failed here, when IV*a* was boiled with formaldehyde and dilute hydrochloric acid, then aerated, a mixture of uroporphyrins resulted, which was shown by paper chromatography to contain octacarboxylic acids only.

[1]*Manuscript received November 23, 1954.*
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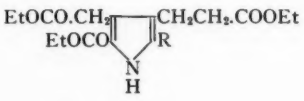
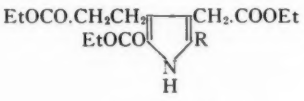
[2]*National Research Council Postdoctorate Fellow.*

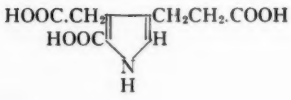
Several analogous chlorinations with sulphuryl chloride in ether, including those of the 3-methyl (5), 3-ethyl (10), and 3-propionic acid (7) derivatives of 2,4-dimethyl-5-carbethoxypyrrole, have not been sufficiently stepwise and complete to give good yields of aldehydes or acids. The influence of impurities in the ether there and in the preparations of I*b* and I*c* is unknown. In the case of II*b*, however, whether or not freshly dried ether was used, when the preliminary drying of the solvent ether was with phosphorus pentoxide rather



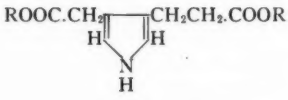
I*a*, R = CH_3
b, R = CHO
c, R = COOH
d, R = H



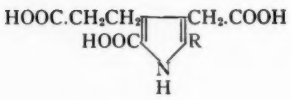
II*a*, R = CH_3
b, R = CHO



III



IV*a*, R = H
b, R = CH_3



V*a*, R = CHO
b, R = CH=N.OH

than with sulphuric acid, the chlorination of II*a* with two moles of sulphuryl chloride was incomplete.[3] The main product, repeatedly isolated after the hydrolysis in 70–90% yield, was 3,3′-di-(carbethoxymethyl)-4,4′-di-(2-carbethoxyethyl)-5,5′-dicarbethoxydipyrromethane. This was identified by melting point and mixed melting point with the methane, m.p. 146°, obtained by boiling II(R = CH_2Br) with water (unpublished).

An improved preparation based on that of Pinner (1, 17, 18) has been worked out for the ethyl acetone-dicarboxylate required in the preparation of I*a*.

EXPERIMENTAL

The ether had been distilled with sulphuric acid and dried over sodium (2). Acetone had been distilled over potassium carbonate, and water had been boiled and cooled under nitrogen.

2-Carboxy-3-(2-carbethoxyethyl)-4-carbethoxymethyl-5-carbethoxypyrrole (*Ic*)

Sulphuryl chloride (10 ml.) was added slowly to a stirred solution of I*a*

[3]*This observation is due to Dr. A. H. Jackson.*

(12.95 gm.) in 200 ml. of absolute ether maintained at $\leqslant 4°$ and protected from moisture. Stirring was continued for 5 min., then for 30 min. with the cooling bath removed. The ether was evaporated *in vacuo* at $\leqslant 20°$, and three 100-cc. portions of ether successively added and evaporated in the same way. Hydrated sodium acetate (40 gm.) in 650 ml. of boiling water was added to the residual oily trichloromethyl derivative, the mixture boiled for three minutes with vigorous stirring and cooled quickly, sodium bicarbonate added to dissolve the precipitate, the solution extracted three times with ether, and the ether washed with 5% sodium bicarbonate (twice with 100 ml.). The combined aqueous layers were filtered, saturated with sulphur dioxide at 20°, and, after one hour at 0°, the crystalline product filtered off and washed with water (twice with 100 ml.). It was dissolved in 500 ml. of boiling ethanol, and water (3.5 liters, heated to 80°) added. After standing overnight the colorless needles were filtered off (8.76 gm., 62%), dried to constant weight over sulphuric acid at 10 mm., m.p. 140–141°, Ehrlich's reaction negative (cold), red (hot). Found in material recrystallized several times from ethanol–water and dried (18 hr., 56°, 0.1 mm.): C, 55.58, 55.62; H, 6.43, 6.25; N, 3.79%. Calc. for $C_{17}H_{23}NO_8$: C, 55.27; H, 6.28; N, 3.47%.

2-Carbethoxy-3-carbethoxymethyl-4-(2-carbethoxyethyl)-pyrrole (Id)

The acid I*c* (3.154 gm.) was heated at 240° under nitrogen until the evolution of carbon dioxide ceased, then sublimed (160–220°, 10 mm.). The sublimate was crystallized three times from ether–hexane to remove oil, giving 590 mgm., m.p. 51–51.5°. An additional 120 mgm. (total, 25%) was obtained from the mother liquors by evaporating, subliming, dissolving in ether, filtering with charcoal, evaporating, putting onto an alumina column in a little methanol, eluting with ether until the cold Ehrlich's reaction was negative, washing the eluate with aqueous sodium bicarbonate, drying it with sodium sulphate, evaporating, and recrystallizing five times from ether–hexane. For analysis, it was recrystallized five times from pentane (Soxhlet), and the colorless plates dried (20 hr., 20°, 10^{-5} mm.), m.p. 51–52°, Ehrlich's reaction bluish-red (cold) (slow). Found: C, 59.29, 59.18; H, 7.14, 7.18; N, 4.60%. Calc. for $C_{16}H_{23}NO_6$: C, 59.06; H, 7.12; N, 4.31%.

2-Carboxy-3-carboxymethyl-4-(2-carboxyethyl)-pyrrole (III)

The ester I*d* (407 mgm.) was refluxed for one hour with 5% potassium hydroxide in 50% ethanol (10 ml.). The cooled solution was put through Amberlite IR–120 (H^+ form, from 20 gm. wet Na^+ form) and eluted with water until the Ehrlich's reaction was negative (cold). The eluate (150 ml.) was concentrated *in vacuo* at *ca.* 20° by swirling on the steam bath until the product crystallized. Filtration and washing with 1 ml. of water gave radiating clusters of thick rods (272 mgm.). A colorless product was obtained after two recrystallizations from acetone (Soxhlet) (143 mgm., 47%), m.p. 178° (decomp.), Ehrlich's reaction bluish-red (cold). For analysis, it was recrystallized four times from acetone and dried overnight (56°, 0.1 mm.). Found: C, 50.08; H, 4.72; N, 6.03%. Calc. for $C_{10}H_{11}NO_6$: C, 49.78; H, 4.60; N, 5.81%.

3-Carboxymethyl-4-(2-carboxyethyl)-pyrrole (Opsopyrrole-dicarboxylic Acid) (IVa)

The pyrrole Ic (0.5 gm.) in 3 ml. of 10% sodium hydroxide was heated under nitrogen in a sealed tube for two hours at 175–180°. The filtered contents of four such tubes were passed through Amberlite IR-120 (H^+ form, from 30 gm. wet Na^+ form), eluting with water (*ca.* 300 ml.) until the Ehrlich's reaction was weak in the cold. The eluate was concentrated (10 mm. *ca.* 20°) by swirling on the steam-bath, then freeze-dried. The colorless residue was extracted into ether (Soxhlet), the ether concentrated to 50 ml., filtered warm, and the product precipitated with hexane as colorless poorly formed needles (882 mgm., 83%), m.p. 139–140°, Ehrlich's reaction bluish-red (cold). For analysis the product was recrystallized four times from ether–hexane and dried (18 hr., 56°, 0.1 mm.). Found: C, 54.79, 54.90; H, 5.69, 5.70; N, 6.94%; eq. wt. 100.3. Calc. for $C_9H_{11}NO_4$: C, 54.81; H, 5.62; N, 7.10%; eq. wt. 98.5.

3-Carbomethoxymethyl-4-(2-carbomethoxyethyl)-pyrrole (Methyl Opsopyrrole-dicarboxylate) (IVb)

Opsopyrrole-dicarboxylic acid (210 mgm.) in ether was allowed to react with excess ethereal diazomethane for 15 min., the ether evaporated, and the residue distilled in a collar flask (138–147° bath temp., 10^{-4} mm.). The yellowish oily product (215 mgm., 90%) was redistilled and the colorless oily middle fraction (135–137° bath temp., 10^{-4} mm.) analyzed, Ehrlich's reaction bluish-red (cold). Found: C, 58.78, 58.67; H, 6.59, 6.39; N, 6.14%. Calc. for $C_{11}H_{15}NO_4$: C, 58.64; H, 6.71; N, 6.22%.

Degradation of Opsopyrrole-dicarboxylic Acid to 3-Methylpyrrole-4-propionic Acid (Opsopyrrole-carboxylic Acid)

The pyrrole IVa (275 mgm.) was heated in a sealed tube for two hours at 150° with 3 ml. of water. The solid left on freeze-drying the combined filtrate and washings from the contents of the tube was extracted with ether (Soxhlet). The ether was concentrated to 20 ml., then replaced by boiling while hexane was added, red amorphous material being filtered off periodically until a slightly cloudy colorless solution was obtained. The product separated, on cooling, as colorless aggregates. After three recrystallizations, 65 mgm. were obtained, m.p. 116°, raised to 117° by sublimation (95–103°, 10^{-2} mm.). Found: C, 62.89, 62.94; H, 7.01, 6.98; N, 9.02%; eq. wt. 148. Calc. for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14%; eq. wt. 153.

Another preparation, after four recrystallizations from chloroform–hexane, had m.p. 115–116°, raised to 118.5–120° after sublimation (Lit.: 119° (not sharp) (11*a*, 4, 7), 117° (8)).

Mixture of Uroporphyrins from Opsopyrrole-dicarboxylic Acid

Opsopyrrole-dicarboxylic acid (100 mgm.) was refluxed for 20 min. in 200 ml. of 0.5% hydrochloric acid and 40 ml. of 1% formaldehyde, cooled, and aerated overnight. The porphyrin was precipitated, filtered off, washed, esterified with methanolic hydrogen chloride, and brought into chloroform in the usual way. The chloroform was washed with 50% aqueous resorcinol (14*b*)

which was subsequently washed with chloroform. The combined chloroform layers were washed free of resorcinol with water, and filtered through a column of alumina (grade V), the porphyrin being eluted with chloroform containing 5% of methanol. After washing with dilute sodium hydroxide and with water, the chloroform was replaced with methanol at the boil, and the solution concentrated to *ca.* 1 ml. giving a mixture of uroporphyrin methyl esters as rods (9 mgm.). Recrystallized six times, it had m.p. 252–258° (4.5 mgm.). Found in 1.5 mgm. sample: C, 60.40; H, 6.84%. Calc. for $C_{48}H_{54}N_4O_{16}$: C, 61.14; H, 5.77%.

A sample was hydrolyzed, and chromatographed on paper with 2,4-lutidine-water in an atmosphere of ammonia (15); the only spot had the same R_f as the uroporphyrin I marker.

Opsopyrrole-dicarboxylic acid did not give porphyrins with chloromethyl ether in alcohol, either on standing or on refluxing (cf. 3) or with formic acid at 100° (cf. 11*a*, 11*b*).

2-Formyl-3-(2-carbethoxyethyl)-4-carbethoxymethyl-5-carbethoxypyrrole (*Ib*)

Sulphuryl chloride (0.477 ml.) was slowly added to the pyrrole I*a* (1 gm.) in 10 ml. of ether, the solution being vigorously stirred, protected from moisture, and kept below 4°. After five minutes, the cooling bath was removed and stirring continued for 30 min. The ether was removed *in vacuo* below 20°, and three 10-ml. portions of ether were successively added and evaporated in the same way. Hydrated sodium acetate (2 gm.) in 50 ml. of boiling water was added to the oily residue of the dichloromethyl derivative, the mixture boiled for three minutes, cooled quickly to 0°, and the crystalline aldehyde filtered off. It was dissolved in 50 ml. of boiling ethanol, and 200 ml. of water (heated to 80°) added. On cooling, the product separated (800 mgm., 77%), m.p. 82–83°. For analysis it was recrystallized three times from ethanol–water and dried (18 hr., 56°, 0.1 mm.) giving nearly colorless needles, m.p. 83.5–84° (Lit.: 84.5° (16)), Ehrlich's reaction red (hot). Found: C, 58.01, 58.00; H, 6.70, 6.39; N, 4.25, 3.88%. Calc. for $C_{17}H_{23}O_7N$: C, 57.76; H, 6.56; N, 3.96%.

2-Formyl-3-carbethoxymethyl-4-(2-carbethoxyethyl)-5-carbethoxypyrrole (*IIb*)

As described for the isomer I*b* above, 10.8 gm. of II*a* (14*a*) in 120 ml. of ether was treated with 5.35 ml. of sulphuryl chloride, the oil evaporated three times with 30 ml. of ether, and boiled with 30 gm. hydrated sodium acetate in 500 ml. of water. The aldehyde was obtained as long needles, m.p. 80–81° (9.87 gm., 88%), after recrystallizing from 500 ml. of ethanol and 2250 ml. of water as for the isomer. For analysis, it was recrystallized five times from ethanol–water and dried (18 hr., 56°, 0.1 mm., nearly colorless needles), m.p. 80.5–81°, Ehrlich's reaction red (hot). Found: C, 57.69, 57.98; H, 6.56, 6.43; N, 4.15%. Calc. for $C_{17}H_{23}NO_7$: C, 57.76; H, 6.56; N, 3.96%.

2-Formyl-3-carboxymethyl-4-(2-carboxyethyl)-5-carboxypyrrole (*Va*)

The corresponding ester II*b* (16 gm.) was heated for two hours with 200 ml. of 10% sodium hydroxide on the steam bath under nitrogen. The cooled solution was passed through Amberlite IR–120 (H^+ form, from 400 gm. wet

Na^+ form) and eluted with water. The eluate (3 liters) was concentrated to 60 ml. (10 mm., bath temp. 60°), and the first crop of the product (10.02 gm.) filtered off. Second and third crops were obtained by concentrating the mother liquors to 20 ml. and filtering (0.37 gm.), then freeze-drying the last liquors. The first crop was extracted into 450 ml. acetone (Soxhlet), the solution concentrated to 150 ml., cooled slightly, and 300 ml. ether added; after colored material was filtered off warm, the first lot crystallized at 0° and was filtered off. The combined second and third crops were treated in the same way with 450 cc. acetone and 300 ml. of ether, colored material separated, and the filtrate combined with the mother liquors from the first lot. Amorphous material was precipitated by adding ether, the solution filtered, and the ether evaporated. The remaining acetone solution was decolorized with charcoal, and two further lots of crystals successively obtained by concentration and cooling. The combined three lots were extracted into 900 ml. of acetone, 2 liters of ether added, and the ether evaporated from the filtered solution while acetone was added to keep the product in solution. After the concentrate was filtered with charcoal, concentrated to 300 ml., and cooled, the product (7.2 gm.) separated as fine needles. The mother liquors were concentrated to 75 ml., giving 1.8 gm. more (total 74%).

For analysis, the product was recrystallized four times from acetone and dried (18 hr., 56°, 0.1 mm.) giving cream-colored needles, decomposing at about 240°, Ehrlich's reaction red (hot). Found: C, 49.45, 49.00; H, 4.22, 4.32; N, 5.02%; eq. wt. 86.3. Calc. for $C_{11}H_{11}NO_7$: C, 49.07; H, 4.12; N, 5.20%; eq. wt. 89.7.

2-Oximinomethyl-3-carboxymethyl-4-(2-carboxyethyl)-5-carboxypyrrole (*Vb*)

A solution of the aldehyde V*a* (8.99 gm.), 8.4 gm. of sodium hydroxide, and 4.7 gm. of hydroxylamine hydrochloride in 500 ml. of water was kept for 18 hr. at room temperature, then heated one and one-half hours on the steam bath. The cooled solution was passed through Amberlite IR-120 (H^+ form, from 200 gm. wet Na^+ form) and eluted with water. The eluate (3 liters) was adjusted to pH 4 with sodium hydroxide and concentrated to 200 ml. on the steam bath under nitrogen at 10 mm., the pH being kept above 2. The first crop, which separated on cooling as radiating needles (6.96 gm.), was recrystallized twice from acetone; a second crop was obtained by concentrating the acetone mother liquors and recrystallizing the solid from acetone. A third crop was obtained by evaporating the aqueous mother liquors to dryness *in vacuo* by swirling on the steam bath, extracting the residue with acetone (Soxhlet), concentrating, extracting the solid which separated with ether (Soxhlet), evaporating the ether until the solution was cloudy, and leaving at 0° overnight. The combined three lots were recrystallized from acetone (Soxhlet) giving 6.64 gm., m.p. 204° (decomp.); concentrating the mother liquor gave 1.68 gm., m.p. 201° (total 86%). For analysis, 120 mgm. were dissolved in 3 ml. acetic acid on the steam bath and 5 ml. of benzol added. The oxime which crystallized after 12 hr. at 0° was recrystallized in the same way giving cream-colored aggregates (17 mgm.), dried (30 hr., 56°, 10^{-3} mm.), m.p. 201° (de-

comp.). Ehrlich's reaction was violet (hot). Found: C, 46.67; H, 4.47; N, 9.75%. Calc. for $C_{11}H_{12}N_2O_7$: C, 46.48; H, 4.26; N, 9.86%.

Although the oxime takes up two moles of hydrogen over palladium black (19) in dilute ammonia, the product has not given consistent analyses.

Ethyl Acetone-dicarboxylate

To 550 gm. of anhydrous citric acid, free of large lumps, in a 5-liter round-bottomed flask protected by a calcium chloride tube, 1100 gm. of 15–18% oleum was added in portions with frequent shaking so that the temperature did not exceed 40°. After standing for three hours at 35–40° with frequent shaking, the solution was cooled below −15° by adding dry ice, and 1 liter of similarly cooled absolute alcohol added in portions with shaking and stirring, the temperature being kept below 0° by adding more dry ice. The mixture was allowed to stand until a clear solution resulted; this required two hours, the temperature rising to 15°. After standing at 0° overnight, the solution was poured into 3.5 liters of ice and water. Three such lots were worked up together. The first was extracted four times with 250 ml. of benzene, the last two extracts being used to extract the second lot, being followed by two extractions with 250 ml. of benzene also used to extract the third lot which was then extracted twice with 250 ml. of fresh benzene. The combined extracts were washed with water, with four lots of 250 ml. of saturated sodium bicarbonate, and with water. After clearing with sodium sulphate and filtering, they were washed twice with 250 ml. of 2% sulphuric acid, four times with 250 ml. of water, cleared with sodium sulphate, filtered, and fractionated through a 12 in. lagged Vigreux column. Yield: 1180 gm. (68%), b.p. (0.8 mm.) 93–96°, η_D^{25} 1.4391.

By the same procedure, except that the initial mixture stood four hours at 40–45°, three lots of 550 gm. of powdered citric acid monohydrate gave 984 gm. (62%) of the ester.

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THE MERCURY PHOTOSENSITIZED HYDROGENATION OF PROPYLENE AND THE ACTIVATION ENERGY OF THE REACTION $C_3H_7+H_2 = C_3H_8+H$ [1]

By G. R. Hoey[2] and D. J. Le Roy

ABSTRACT

The reactions initiated in hydrogen–propylene mixtures by $Hg(^3P_1)$ atoms were studied over the temperature range from room temperature to 320°C. At 260° and above, the rate of formation of propane and the rate of pressure decrease are linear functions of the hydrogen pressure. This effect is attributed to the reaction $C_3H_7+H_2 = C_3H_8+H$ and its activation energy is estimated to be equal to or slightly greater than 12.5 kcal. per mole. This is 1.2 kcal. per mole greater than the corrected value for the activation energy of the analogous reaction $C_2H_5+H_2 = C_2H_6+H$. The ratio $k_{combination}/k_{disproportionation}$ is estimated to be approximately 2.0 at room temperature in the case of isopropyl radicals.

INTRODUCTION

Kinetic data are now available for the reaction of methyl (10, 17, 14) and ethyl (18, 9) radicals with hydrogen as well as for the reverse reactions of atomic hydrogen with methane (2) and ethane (1). The present investigation was undertaken for the purpose of obtaining data on the reaction of propyl radicals with hydrogen,

$$C_3H_7+H_2 = C_3H_8+H.$$

The method chosen was similar to that used by Le Roy and Kahn (9) in their study of the analogous reaction,

$$C_2H_5+H_2 = C_2H_6+H. \qquad [a]$$

Their method can be described briefly.

At temperatures below approximately 200°C. the mercury ($^3P_1$) photosensitized hydrogenation of ethylene was interpreted in terms of the following reactions:

$$Hg(^3P_1)+H_2 = 2H+Hg(^1S_0) \qquad [b]$$

$$H+C_2H_4 = C_2H_5 \qquad [c]$$

$$2C_2H_5 = C_4H_{10} \qquad [d]$$

$$2C_2H_5 = C_2H_6+C_2H_4. \qquad [e]$$

In agreement with this mechanism, the rate of pressure decrease was independent of hydrogen pressure. At high temperatures, however, the rate of pressure decrease and the rate of formation of ethane increased linearly with the hydrogen concentration. This effect was attributed to reaction [*a*]. When this reaction is incorporated into the mechanism the predicted slope of the *rate* vs. (H_2) curve is proportional to $k_a/(k_d+k_e)^{\frac{1}{2}}$. From the effect of tempera-

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ture on the slope Le Roy and Kahn obtained a minimum value of 10.5 kcal. per mole for E_a by neglecting the effect of temperature on $(k_d+k_e)^{\frac{1}{2}}$. Their result was in good agreement with the value of 11.5±1 kcal. per mole subsequently obtained by Wijnen and Steacie (18) from experiments on the photolysis of diethyl ketone in the presence of deuterium.

Moore and Taylor (12), in their investigation of the mercury photosensitized hydrogenation of propylene, confined their measurements to room temperature. Moore (11) later studied the reaction at temperatures up to 200°C., but he did not investigate the effect of hydrogen pressure. In the present investigation we have studied the effect of hydrogen pressure on the mercury photosensitized hydrogenation of propylene over the temperature range from room temperature to 320°C. and we have been able to apply the method of Le Roy and Kahn to obtain a minimum value for the activation energy of the reaction of isopropyl radicals with hydrogen.

EXPERIMENTAL

Two different types of reaction system were used. The first (I) involved a quartz annular cell and furnace and a lamp arrangement similar to that used previously (9). Pressures were measured on a wide bore U-tube manometer. This apparatus was used to obtain a considerable fund of analytical data for 300° and 320°C. but it was not suitable for accurate pressure measurements or for reproducible light intensities.

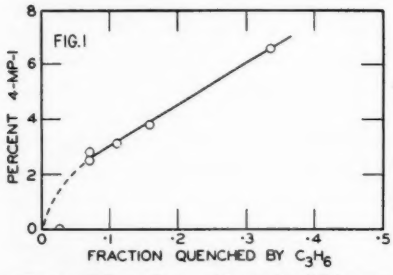
The second reaction system (II) was particularly well adapted for the determination of rates at constant incident light intensity. The cylindrical quartz cell, 5 cm. in diameter and 10 cm. long, had a plane window at one end; the other end of the cell was tapered down to a quartz-to-pyrex graded seal which was sealed to the inner member of a 19/38 standard taper joint. During an experiment gas was circulated through the cell by a mercury piston pump; the gas entered through a 6 mm. tube ring-sealed to the outer part of the 19/38 joint and extending to within a few millimeters of the window. The cell was surrounded by a close fitting aluminum block furnace with a quartz window placed about 7.5 cm. beyond the cell window. The upper half of the furnace could be taken off to remove and clean the cell. The temperature of the furnace was controlled to within ±0.5°C. The lamp used in connection with this cell was of the usual type with rare gas carrier, but since it was entirely outside the heated zone its output was independent of the reaction temperature. With this reaction system more accurate pressure measurements were made possible by using a modified form of the differential manometer described previously (7) with a multiplication ratio of approximately nine. Of the total volume of the system 64.2% was outside the furnace.

Commercial electrolytic hydrogen was purified by passage through platinized asbestos at 500°C. and through silica gel at the temperature of liquid air. The propylene (Ohio Chemical Company) was said to have a purity of 99.5%; it was subjected to several trap-to-trap distillations before being placed in the storage reservoir. The hydrocarbons used for infrared identification were standard samples obtained from the United States Bureau of Standards.

The products were separated into fractions by low temperature distillation (8). The methane in the non-condensable fraction was separated from hydrogen by diffusion of the latter through palladium (8). The olefin content of the C_2, C_3, and C_4 fractions was determined by the method of Pyke, Kahn, and Le Roy (13). Infrared spectra of the C_5 and C_6 fractions were taken. Insufficient C_5 material was obtained for identification except at high temperatures; experiments at 300°C. showed this fraction to contain 2-methyl butene-1 to the extent of approximately 40%, the remainder was an unidentified paraffin. The C_6 fraction was found to contain 2,3-dimethyl butane and 4-methyl pentene-1, as well as an unidentified material comprising, on the average, about 8% of the fraction. No evidence was found for *n*-hexane or 2-methyl pentane; this is in agreement with the observations of Moore (11) and suggests that only isopropyl radicals are formed by the addition of atomic hydrogen to propylene.

RESULTS

A series of experiments was done at room temperature, using cell II, for the purpose of identifying products and studying their possible dependence on hydrogen pressure. In these experiments, and in those described subsequently, the pressure drop was not allowed to exceed 50% of the initial propylene pressure; preliminary experiments had shown that the rate of pressure drop was constant until the pressure drop reached approximately 75% of the initial propylene pressure. For propylene pressures from 10 to 53.6 mm. and hydrogen pressures from 115 to 380 mm. the proportions of the various products, with the exception of 4-methyl pentene-1, were independent of the initial composition of the reaction mixture within the accuracy of the measurements. The average composition of the products was: 2,3-dimethyl butane 46.0%, propane 36.6%, methane 4.9%, unidentified C_6 fraction 4.5%, C_5 2.3%, butane 1%, butene 1%.



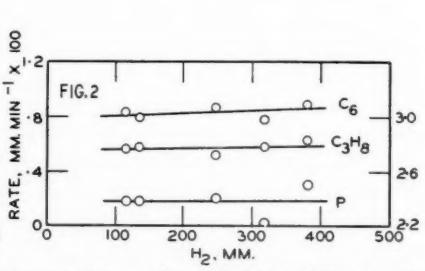


FIG 1. Effect of quenching of Hg($^3P_1$) atoms by propylene on the yield of 4-methyl pentene-1.
FIG. 2. Effect of hydrogen pressure on the rate of formation of the C_6 fraction and of propane (left hand scale) and on the rate of pressure decrease (right hand scale) at room temperature. Initial pressures of propylene were from 9.9 to 27.8 mm.

The percentage of 4-methyl pentene-1 increased considerably with the ratio of propylene to hydrogen. In Fig. 1 this percentage is plotted against the fraction of Hg($^3P_1$) atoms quenched by propylene, F. The value of F was calculated from the average concentrations of propylene and hydrogen, the

molecular weights of these two gases and of mercury, and the quenching cross sections for collisions of the two gases with $Hg(^3P_1)$ atoms. The quenching cross section for propylene was assumed to be 50×10^{-16} cm.^{2}, i.e. slightly greater than the value found by Steacie (16) for ethylene. The value taken for hydrogen was 8.9×10^{-16} cm.^{2} (16). Since only the ratio of these two quantities is involved in the calculation of F, the numbers would not be changed appreciably by using Darwent's suggested values of 31.0×10^{-16} and 6.0×10^{-16} for propylene and hydrogen, respectively (5).

The form of the curve in Fig. 1 suggests that 4-methyl pentene-1 probably arises through the quenching of $Hg(^3P_1)$ atoms by propylene. No 4-methyl pentene-1 could be detected in the experiment for which F was 0.028 (initial propylene 10 mm., initial hydrogen 380 mm.), which affords some justification for extrapolating the curve through the origin.

The rate of production of C_6 and propane and the rate of pressure drop are shown as functions of the hydrogen pressure in Fig. 2. This graph does not include the data for the experiment for which F was equal to 0.336 (*vide* Fig. 1); because of the considerable amount of quenching by propylene in this experiment the rate of pressure drop was only 1.68×10^{-2} mm. min.$^{-1}$.

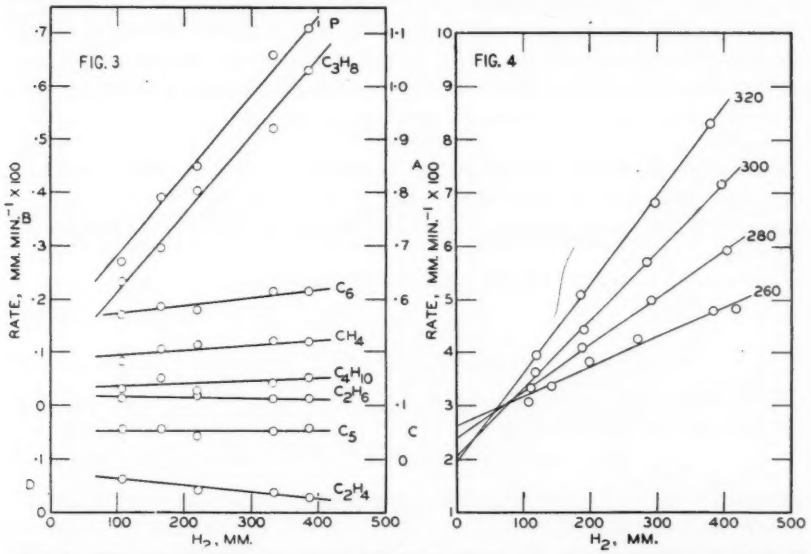


FIG. 3. Effect of hydrogen pressure at 320°C. Scale A: rate of pressure decrease; Scale B: rate of formation of methane, ethane, propane, butane, and C_6; Scale C: rate of formation of C_6; Scale D: rate of formation of ethylene. Initial pressures of propylene were in the range 17.7 to 18.2 mm.

FIG. 4. Effect of hydrogen pressure on the rate of pressure decrease at 260°, 280°, 300° and 320°C., using system II. In most cases the ratio hydrogen : propylene was of the order of 20 : 1 or greater. The ordinates refer to pressures corrected to 25°C., the abscissae to observed pressures at the reaction temperature.

In Fig. 3 are shown the results of a series of experiments using cell I at 320°C. The rate of production of propane and the rate of pressure drop are strongly

dependent on the hydrogen pressure at this temperature, while the rates of formation of the other products are not. Also, the rates of formation of ethane and ethylene are appreciable at 320°, although neither of these products could be detected at 25°C. A small amount of a product higher than C_6 was found at 320°C.; its rate of formation was independent of hydrogen pressure and equal to approximately 0.02×10^{-2} mm. min.$^{-1}$. At 25°C. the rates of formation of butane and butene were the same; at 320°C. the rate of formation of butene (*ca.* 0.012×10^{-2} mm. min.$^{-1}$) was considerably less than that of butane.

Quantitative comparison of the rates of formation of the various products at the two temperatures cannot be made with certainty because different lamps and cells were used. However, relative to the rate of formation of C_6, the rate of formation of methane is 6.3 times as great and of butane 11.5 times as great at 320° as at 25°C. The ratio of C_6 to propane for "zero hydrogen pressure" is 1.5 for 25°, 2.4 for 320°C. Since the C_6 fraction is largely 2,3-dimethyl butane, and since reaction [5] would not be expected to occur at low hydrogen pressures (see below) it would appear that there is little difference in activation energy for the combination and disproportionation of isopropyl radicals.

When a correlation had been established between the rate of pressure drop and the rate of formation of propane at high temperatures, a series of experiments was done to determine with some precision the rate of pressure drop as a function of hydrogen pressure and temperature. These measurements were made with cell II and the differential manometer; the results are shown in Fig. 4.

DISCUSSION

Even at the highest temperatures used, propane and 2,3-dimethyl butane are the major products. In view of the results shown in Figs. 2 and 3 it would appear that the most important reactions leading to their formation are the following:

$$Hg(^3P_1)+H_2 = 2H+Hg(^1S_0) \qquad [1]$$

$$H+C_3H_6 = C_3H_7 \qquad [2]$$

$$2C_3H_7 = C_6H_{14} \qquad [3]$$

$$2C_3H_7 = C_3H_8+C_3H_6. \qquad [4]$$

$$C_3H_7+H_2 = C_3H_8+H. \qquad [5]$$

From the effect of hydrogen pressure on the rate of formation of propane it is clear that reaction [5] is negligible at room temperature but of increasing importance as the temperature is raised. Although at higher temperatures the rates of formation of methane and butane are greater, and ethane and ethylene appear in appreciable quantities, the formation of these products does not seem to be strongly influenced by hydrogen pressure. Furthermore, in the experiments at 320°C. in which propane was measured there was a good correlation between the rate of formation of propane and the rate of pressure drop. It therefore seems justifiable to interpret the slopes of the curves in Fig. 4 as showing the effect of hydrogen pressure on the rate of formation of propane.

Since, to a good approximation, all the products are saturated it follows that the rate of consumption of propylene will be almost identical with the rate of pressure drop.

The basic mechanism, reactions [1] to [5], inclusive, will be referred to as *I*. According to *I* the rate equation for the consumption of propylene is

$$-d(C_3H_6)/dt = I_a\left\{1+\frac{k_3}{k_3+k_4}\right\}+\frac{k_5 I_a^{\frac{1}{2}}}{(k_3+k_4)^{\frac{1}{2}}}(H_2). \qquad [i]$$

The curves in Fig. 4 satisfy an equation of the same form as [*i*], although mechanism *I* takes no account of the formation of methane, ethylene, or butane, to mention only three of the additional products found at higher temperatures. Also, mechanism *I* does not allow for the instability of the propyl radical at high temperatures (4) or for the possible occurrence of reaction [6],

$$H+C_3H_7 = C_3H_8. \qquad [6]$$

The analogous reaction between H atoms and ethyl radicals has been shown to be of some importance, under certain conditions, in the mercury photosensitized hydrogenation of ethylene (15).

If reaction [6], which may involve a third body, is included it is found that [*i*] still applies provided $k_2(k_3+k_4)(C_3H_6)/(k_5k_6(H_2))$ is appreciably greater than unity. From a consideration of the probable magnitudes of the quantities involved and from our analogous experiments with ethylene (15), it would appear that this condition obtains.

It is easily shown that if the propyl radical decomposes according to reaction [7],

$$C_3H_7 = C_3H_6+H, \qquad [7]$$

this will have no effect on the validity of [*i*].

If the propyl radical decomposes according to reaction [8],

$$C_3H_7 = C_2H_4+CH_3, \qquad [8]$$

and this is followed by [9],

$$CH_3+H_2 = CH_4+H, \qquad [9]$$

methane and ethylene will be formed at the same rate, viz. $k_3 I_a^{\frac{1}{2}}/(k_3+k_4)^{\frac{1}{2}}$ and a term of this same magnitude will be *added* to [*i*]; for brevity this term will be referred to as *A*.

If [8] is followed by [10],

$$CH_3+C_3H_7 = C_4H_{10}, \qquad [10]$$

it can be shown that [*i*] will be valid provided $(2I_a/A)^2$ is appreciably greater than unity. This condition undoubtedly obtains at the temperatures and light intensity used. Ethylene and butane will each be formed at a rate equal to *A*.

If [8] is followed by [11],

$$CH_3+H = CH_4, \qquad [11]$$

and if $(2I_a/A)^2$ is appreciably greater than unity it can be shown that the term *A* must be *subtracted* from [*i*]. Methane and ethylene will then each be formed at the rate *A*.

In all of the above cases the mechanisms predict that the steady state H atom concentration should increase with the concentration of H_2. The secondary reaction,

$$H+C_2H_4 = C_2H_5, \qquad [12]$$

would therefore be expected to become more important at high pressures of hydrogen. This probably accounts for the decrease in the yield of ethylene shown in Fig. 3.

Reactions [1] to [11] account in an adequate manner for all of the major products. In addition, some butane will be formed by the combination of ethyl radicals formed in [12]. The 4-methyl pentene-1, formed at low hydrogen concentrations, undoubtedly arises by the combination of propyl radicals with allyl radicals formed in the quenching of propylene by $Hg(^3P_1)$ atoms.

Bywater and Steacie (4) considered reaction [13],

$$H+C_3H_7 = CH_3+C_2H_5, \qquad [13]$$

to be the source of methyl radicals in the mercury photosensitized decomposition of propane at temperatures below 300°C. This reaction could occur if the excited propane formed in [6] were not deactivated. Reaction [6] would be more probable than [13] at the pressures used in these experiments, and since it has been shown that the occurrence of [6] is not likely to affect the validity of [*i*] it seems justifiable to interpret the slopes of the curves in Fig. 4 in terms of [*i*].

The logarithms of the slopes of the curves in Fig. 4 are plotted against $1000/T$ in Fig. 5. Least squares plots were used in each case. Since the ordinates in Fig. 4 refer to pressures measured with the whole system at 298°K., and the abscissae to pressures measured with the cell at the reaction temperature, T°K., it follows that the slopes, S, are given by the expression

$$S = \{298 V_c k_5 I_a^{\frac{1}{2}}\}/\{T(V_c+V_b)(k_3+k_4)^{\frac{1}{2}}\}, \qquad [ii]$$

in which V_c is the volume of the cell and V_b that of the rest of the system. Hence,

$$E_5 = -R\, d\{\ln S+\ln(k_3+k_4)^{\frac{1}{2}}+\ln T\}/d(1/T). \qquad [iii]$$

From Fig. 5 the first term is 11.4 kcal. per mole. The second term will be

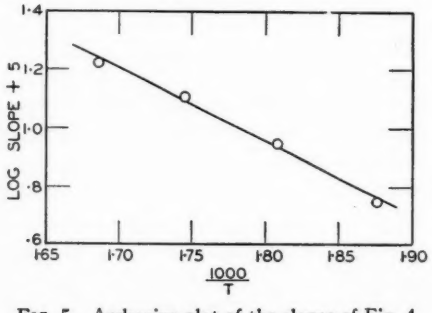


FIG. 5. Arrhenius plot of the slopes of Fig. 4.

neglected; the third is equal to RT. Using the average reaction temperature, 290°C., $E_5 = 12.5$ kcal. per mole.

For the analogous reaction,

$$C_2H_5+H_2 = C_2H_6+H \qquad [14]$$

Le Roy and Kahn found $-R\,d\ln S/d(1/T)$ to be 10.5 kcal. per mole (9). However, they plotted values of $-dP/dt$ reduced to 25°C. against values of P_H, reduced to 25°C. In this case the third term is $RT^2V_b/(298V_c+TV_b)$, rather than RT. In their experiments V_c/V_b was 0.87, and for an average reaction temperature of 290°C. the correction term is 0.8 kcal. per mole. The corrected value of E_{14} is then 11.3 kcal. per mole, in good agreement with the value 11.5±1 kcal. per mole obtained by Wijnen and Steacie (18).

The errors introduced by neglecting the effect of temperature on combination and disproportionation in evaluating E_5 and E_{14} are probably less than 1 kcal. per mole.

An estimate of the value of k_3/k_4 may be obtained from the present data. The experiments of Moore and Taylor (12) suggest a value of 2.5 for this quantity at room temperature. Blacet and Calvert (3) and Durham and Steacie (6) found it to be *ca.* 2.0 at room temperature, while the latter obtained a value of 2.7 at 121°C. If we equate the ratio of hexane to propane at "zero hydrogen pressure" to k_3/k_4 the present results yield the value 1.3 at room temperature, *ca.* 2.1 at 320°C. The increase with temperature is in line with the results of Durham and Steacie. However, our experiments with ethylene (15) have shown that (product of combination)/(product of disproportionation) only approaches $k_{comb.}/k_{disp.}$ when the concentration of olefin is high, because of the influence of reaction [6] or its analogue. Moore and Taylor used a propylene pressure of 40 mm., which should be sufficient to yield a good value of k_3/k_4, but the ratio of propylene to hydrogen was only 1:6 which, in our experience, would result in the presence of a considerable amount of 4-methyl pentene-1 in the C_6 fraction. Their value of 2.5 at room temperature is probably too high for this reason. Our value is probably too low. A value close to 2.0 at room temperature would therefore seem to be established for the ratio of the rate of combination to the rate of disproportionation of isopropyl radicals.

ACKNOWLEDGMENT

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COMPRESSIBILITY OF GASES AT HIGH TEMPERATURES

IX. SECOND VIRIAL COEFFICIENTS AND THE INTERMOLECULAR POTENTIAL OF NEON[1]

By G. A. Nicholson[2] and W. G. Schneider

ABSTRACT

The second virial coefficients of neon have been determined in the temperature range 0° to 700°C. and the pressure range 10 to 80 atmospheres. These data were combined with published low temperature (−150° to 0°C.) second virial data, to investigate the intermolecular potentials of neon using both a Lennard-Jones potential, with a 9th and 12th power repulsion term, and also a modified Buckingham exponential–six potential. The agreement between observed and calculated values of $B(T)$ was excellent for both the exponential–six and the Lennard-Jones 12:6 potentials and slightly less satisfactory for the Lennard-Jones 9:6 potential.

VIRIAL COEFFICIENT DETERMINATION

Introduction

The determination of the second virial coefficients of neon consisted essentially of the accurate measurement of the pressure of gas in a pipette followed by the expansion of the gas into a smaller pipette, both pipettes being maintained at constant temperature, and the measurement of the final pressure. A series of experiments were carried out with different initial pressures and at different temperatures, and from the data obtained it was possible to calculate the virial coefficient at any of the experimental temperatures. Full details of the method have already been described in previous papers (10, 11, 14, 6).

Experimental

Stainless steel pipettes with volumes of approximately 220 and 50 ml. were used. Both vessels were maintained at constant temperature in a thermostat: oil was used as thermostat liquid up to 100°C. and a molten eutectic mixture of lithium, potassium, and sodium nitrates for temperatures above 100°C. Temperatures were controlled automatically to ±0.005°C. or better, using a photocell–amplifier–relay circuit in conjunction with a platinum resistance thermometer. A second platinum resistance thermometer was used to measure the temperature of the thermostat liquid.

Pressure measurements were made with a Keyes type of dead weight piston gauge which had been calibrated against the vapor pressure of carbon dioxide at 0°C. This instrument and the corrections to be applied to the measured pressure have already been described (10).

The neon used in these determinations was supplied by Linde Air Products as spectroscopically pure neon. Mass spectroscopic examination of the gas did not reveal any impurities, and subsequent analyses carried out at the beginning and end of each isotherm confirmed this.

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[2]*National Research Council of Canada Postdoctorate Fellow, 1953–54.*

Isotherms of neon were determined at 0°, 50°, 100°, and at 100° intervals up to 700°C. From these data second virial coefficients and intermolecular potentials have been calculated.

Interpretation of Experimental Results

If both pipettes are at a temperature T, their respective volumes are V_1 and V_2, and the number of molecules present initially in the large pipette at a pressure P_1 is n, then since we are dealing with a permanent gas at high temperatures and relatively low pressures, the isotherms can be fitted to simple virial expressions of the form

$$P_1V_1/n = A_T+B_TP_1+C_TP_1^2. \quad [1]$$

On expanding the gas into the second pipette, the pressure is reduced to P_2 but n remains constant

$$P_2(V_1+V_2)/n = A_T + B_TP_2 + C_TP_2^2. \quad [2]$$

Combining equations [1] and [2] and eliminating n we get

$$\frac{P_1}{P_2} = N+(N-1)\frac{B_T}{A_T}P_1+(P_1N-P_2)\frac{C_T}{A_T}P_1 \quad [3]$$

where $N = (V_1+V_2)/V_1$.

The isotherms of neon are linear above 50°C. and the contribution due to the term involving the third virial coefficient can therefore be neglected in equation [3]. It must be considered, however, in calculating virial data for the 0° and 50°C. isotherms because these isotherms were found to have a slight curvature.

Considering the simple case

$$\frac{P_1}{P_2} = N+(N-1)\frac{B_T}{A_T}P_1, \quad [4]$$

N is the intercept when the pressure ratio P_1/P_2 is plotted against P_1 and the value of B_T/A_T can therefore be derived from the slope of the graph. In order to calculate B_T for each isotherm, A_T must be known. If A_T and B_T are expressed in amagat units, then for the 0°C. isotherm

$$A_0+B_0 = 1.$$

Thus A_0 and B_0 can be determined and A_T is then calculated from the expression

$$A_T = A_0T/273.16.$$

Results

Experimental results are shown in Fig. 1 where the pressure ratio, P_1/P_2 is plotted against the pressure in the large pipette before expansion, P_1.*

**It should be noted that the experimental pressures P_1 along the abscissa are not equally spaced, but become progressively more closely spaced at lower pressures, approximately in the ratio $1/N$, as is obvious from equation [4]. This crowding together of the experimental points at low pressures is of great advantage in determining the limiting slope of the curve since from it the second virial coefficient is derived.*

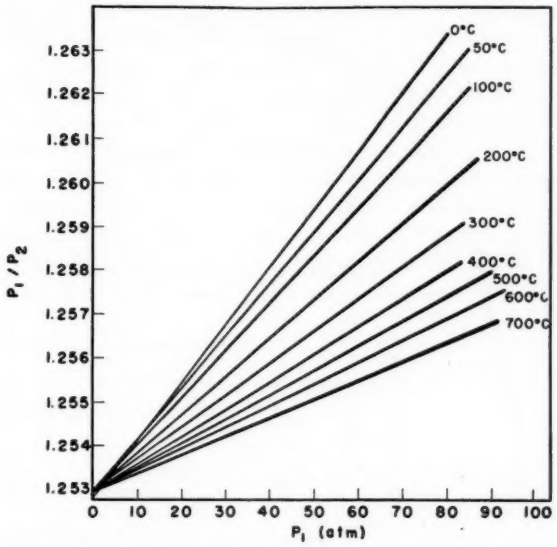

FIG. 1. Plot of pressure-ratios, P_1/P_2, vs. pressure, P_1, for neon.

The scatter of the individual points about the lines would not be noticeable on this scale and so all experimental points have been omitted. In every case, the scatter obtained was of the same order as the accuracy of the pressure measurement. At each temperature at least three expansion runs were carried out, each run usually consisting of eight expansions. The best line to represent the experimental results for each isotherm was fitted by a method of least mean squares (12) and the virial coefficients obtained from these are given in Table I. These data, expressed in amagat units of volume, are converted to cm.^{3}/mole units by multiplying by the normal volume V_N where

$$V_N = 22414.6/A_0 \text{ cm.}^3$$

The variation of the second virial coefficient, B, with temperature is shown graphically in Fig. 2. This graph also shows the results of other measurements carried out on neon (3, 4, 8, 5) and the data are recorded in Table II. There is

TABLE I

VIRIAL DATA AT DIFFERENT TEMPERATURES
(amagat units)

Temperature (°C.)	A_T	B_T ($\times 10^3$)	C_T ($\times 10^6$)
0	0.999514	0.4865	0.1996
50	1.182467	0.5494	0.0459
100	1.365375	0.5690	
200	1.731270	0.5987	
300	2.097165	0.6107	
400	2.463061	0.6143	
500	2.828956	0.6125	
600	3.194851	0.6192	
700	3.560747	0.6235	

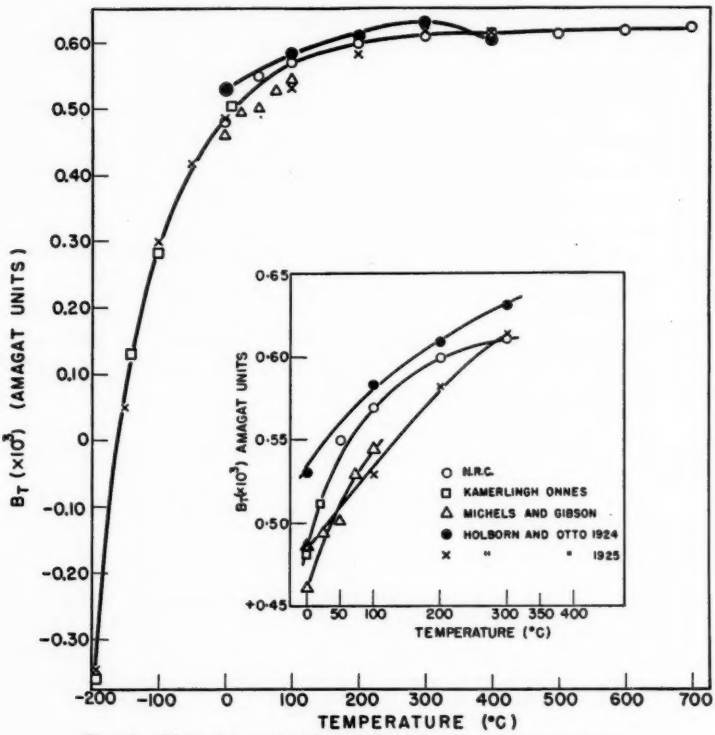

FIG. 2. Plot of second virial coefficients, B_T, vs. temperature.

TABLE II

COMPARISON OF MEASURED SECOND VIRIAL COEFFICIENTS WITH PREVIOUS VIRIAL DATA (×10³) (AMAGAT UNITS)

Temp. (°C.)	N.R.C.	Michels and Gibson	Holborn and Otto 1924	Holborn and Otto 1925	Kamerlingh-Onnes *et al.*
−182.5	—	—	—	—	−0.362
−150.0	—	—	—	0.055	—
−141.22	—	—	—	—	0.130
−103.01	—	—	—	—	0.281
−100.0	—	—	—	0.301	—
−50.0	—	—	—	0.418	—
0.0	0.4865	0.4612	0.5302	0.486	0.481
20.0	—	—	—	—	0.5114
24.47	—	0.4940	—	—	—
50.0	0.5494	—	—	—	—
50.40	—	0.5017	—	—	—
72.83	—	0.5292	—	—	—
100.0	0.5690	—	0.5834	0.529	—
100.83	—	0.5429	—	—	—
200.0	0.5987	—	0.6090	0.582	—
300.0	0.6107	—	0.6308	0.614	—
400.0	0.6143	—	0.6071	0.613	—
500.0	0.6125	—	—	—	—
600.0	0.6192	—	—	—	—
700.0	0.6235	—	—	—	—

good agreement between all the second virial data for neon except those results obtained in the temperature range 0° to 200°C. The data of Michels, Kamerlingh-Onnes, Holborn, and Otto (4), and N.R.C. agree quite closely at 0°C. but the Holborn and Otto (3) result is much higher. However, all the other Holborn and Otto (3) results are in fairly good agreement with N.R.C. results, while those of Michels, Kamerlingh-Onnes, and Holborn and Otto (4) in the temperature range 25° to 200°C. are much lower. It is interesting to note the sudden changes of curvature of the line joining the results of Holborn and Otto (4) at about 0°C. and 200°C. There appears to be no apparent explanation for the discrepancies in the data over the range 0° to 200°C. although the presence of impurities in the gas may be one of the most probable reasons.

ON THE INTERMOLECULAR POTENTIAL OF NEON

The second virial coefficient is related to the energy of interaction between pairs of molecules by the expression

$$B(T) = 2\pi N \int_0^\infty r^2(1-e^{-E(r)/kT})\,dr$$

where $B(T)$ is the second virial coefficient,

N is Avogadro's number,

$E(r)$ is the potential energy of interaction as a function of distance r.

Using this expression it is easy to calculate values for the second virial coefficients provided the potential $E(r)$ is known. The reverse process is not so easy, however, unless a fairly simple expression is used for the term $E(r)$. One such expression is the Lennard-Jones inverse power potential:

$$E(r) = 4\epsilon[(r_0/r)^n - (r_0/r)^6],$$

the special case of this being when $n = 12$, i.e. a 12th power of repulsion and a sixth power of attraction. In the above expression ϵ is the energy at the minimum of the potential energy curve and r_0 is the low-velocity collision diameter. The value of r at the minimum will be designated as r_m. An exp: 6 intermolecular potential

$$E(r) = \frac{\epsilon}{1-6/\alpha}\left[\frac{6}{\alpha}e^{\alpha(1-r/r_m)} - \left(\frac{r_m}{r}\right)^6\right],$$

where ϵ is the depth of the potential energy minimum,

r_m is the position of the minimum,

α is a parameter which measures the steepness of the repulsion energy, has also been used successfully, and it has also been used to predict data on transport properties with fair accuracy.

The experimental results were fitted with a Lennard-Jones 12:6 and 9:6 potential and also an exp:6 potential. For the Lennard-Jones 12:6 potential, integration of the above expression for B leads to $B(T) = b_0\,\beta(\tau)$ where $b_0 = \frac{2}{3}\pi N r_0^3$ and $\tau = kT/\epsilon$. Tables for the function $\beta(\tau)$ have been compiled by Hirschfelder, Bird, and Spotz (9). A method of least mean squares (13) was

used to calculate the best potential parameters. Two sets of virial data were used:

(i) N.R.C. data only.

(ii) N.R.C. data together with the low temperature (0° to −150°C.) data of Holborn and Otto (4) and Kamerlingh-Onnes. Quantum mechanical corrections (2) were applied to all virial data at temperatures of 0°C. and lower before they were used in the calculations. All values shown in Table III have already been corrected.

Good agreement was obtained between the two sets of data for the potential parameters (see Table III) and therefore in subsequent calculations, N.R.C.

TABLE III

COMPARISON OF EXPERIMENTAL AND CALCULATED SECOND VIRIAL COEFFICIENTS

		Lennard-Jones potential				Buckingham exp : 6 potential	
		12 : 6		9 : 6			
		r_0 2.756 Å r_m 3.093 Å ϵ/k 33.74°K. ± 0.18 ϵ 46.55 × 10^{-16} erg		r_0 3.326 Å r_m 3.806 Å ϵ/k 25.42°K. ± 0.01 ϵ 35.07 × 10^{-16} erg		r_0 2.758 Å r_m 3.084 Å ϵ/k 37.36°K. ± 0.19 ϵ 51.55 ± 10^{-16} erg α 15	
Temp. (°K.)	Exptl. 2nd virial coefficient ($cm.^3$/mole)	Second virial coefficient ($cm.^3$/mole)					
		Calc.	Exp. − calc.	Calc.	Exp. − calc.	Calc.	Exp. − calc.
123.16	1.23	1.36	−0.13	1.31	−0.08	1.30	−0.07
131.94	2.92	2.65	+0.27	2.66	+0.26	2.62	+0.30
170.15	6.30	6.54	−0.24	6.68	−0.38	6.59	−0.29
173.16	6.75	6.77	−0.02	6.90	−0.15	6.81	−0.06
223.16	9.37	9.46	−0.09	9.66	−0.29	9.52	−0.15
273.16	11.12	11.07	+0.11	11.21	−0.09	11.09	+0.03
323.16	12.32	11.97	+0.35	12.17	+0.15	12.05	+0.27
373.16	12.76	12.61	+0.15	12.77	−0.01	12.68	+0.08
473.16	13.43	13.35	+0.08	13.44	−0.01	13.39	+0.04
573.16	13.70	13.71	−0.01	13.72	−0.02	13.70	0.00
673.16	13.78	13.88	−0.10	13.83	−0.05	13.86	−0.08
773.16	13.74	13.96	−0.22	13.84	−0 10	13.92	−0.18
873.16	13.89	13.97	−0.08	13.79	+0.10	13.91	−0.02
973.16	13.98	13.94	+0.04	13.72	+0.26	13.85	+0.13

data were combined with the low-temperature data of previous workers. Second virial coefficients were calculated from the potential parameters and these are compared with experimental values in Table III. It is apparent from the comparison that the deviations for the 12:6 potential and the exp:6 potential are very similar and neither potential appears to be superior to the other. This is perhaps not surprising in view of the close similarity of the two types of potential energy curves, which are shown plotted in Fig. 3. The 9:6 potential gives a slightly poorer fit to the experimental data in that the sum of squares of the deviations is somewhat higher than for the other two potentials. The over-all fit is nevertheless surprisingly good in view of the rather large difference of this potential (also shown in Fig. 3) compared with the 12:6 and exp:6 potentials.

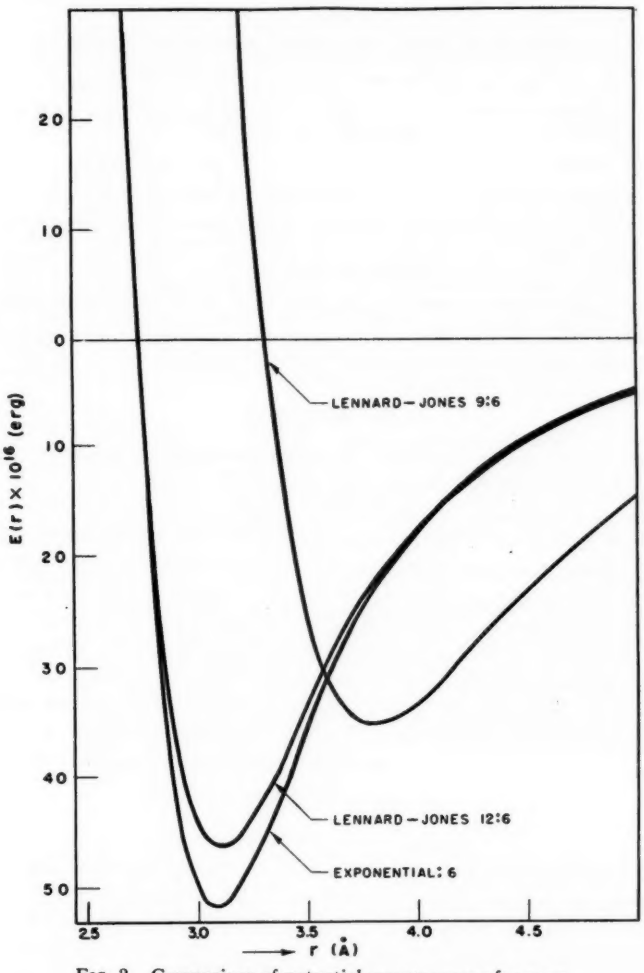

FIG. 3. Comparison of potential energy curves for neon.

Corner (1) has shown recently how calculations of intermolecular potentials may be improved by the use of crystal data in addition to gas property data. He used this method to determine the potentials of neon with repulsion of an exponential type from crystal, second virial, and Joule-Thomson coefficient data. Mason and Rice (7) extended the application of Corner's method to include experimental transport property results for the evaluation of the intermolecular potentials of a number of simple non-polar molecules, including neon. For comparison, the potential parameters obtained by Corner (1) are given in Table IV together with those of Mason and Rice (7) and N.R.C.: these are for an exp:6 potential only. The over-all agreement is very satisfactory.

TABLE IV

COMPARISON OF POTENTIAL PARAMETERS FOR AN EXP : 6 POTENTIAL

	α	r_m, Å	ϵ/k, °K.
N.R.C.	15	3.084	37.36
Corner	13.6	3.16	37.1
Mason and Rice	14.5	3.147	38.0

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THE DEGRADATION OF CARRAGEENIN

I. KINETICS IN AQUEOUS SOLUTION AT pH 7[1]

By C. R. Masson

ABSTRACT

The degradation of carrageenin in buffered aqueous solution at pH 7.0 has been studied over the temperature range 60° to 101°C. by following the change in viscosity and reducing properties. Kinetic analysis indicates the occurrence of two reactions: (*a*) an initial rapid degradation representing only about 0.3% of the complete hydrolysis, followed by (*b*) a first-order random degradation having $k = 2.75 \times 10^{13}\, e^{-29,200/RT}$ hr^{-1}. The latter reaction becomes important at temperatures above 60°C. The results indicate that carrageenin is more unstable than would be expected of a simple 1–3 galactan, and that two types of weak linkage are involved in the structure of the polysaccharide.

INTRODUCTION

The polysaccharide carrageenin is a well known naturally-occurring hydrocolloid which, by virtue of its gelling and stabilizing properties in aqueous solution, is of commercial importance (12). Black (1) has recently reviewed the literature on the structure of this polysaccharide. The main units are D-galactose residues joined through carbon atoms 1 and 3 and carrying a half-ester sulphate group on carbon 4. The polysaccharide is obtained by extracting with water the red alga *Chondrus crispus*. In the commercial process, heat is generally employed at some stage during extraction and drying, but, although it has long been known (3) that carrageenin solutions are thermally unstable, no systematic study of the degradation has been reported. Such a study might not only yield results of practical value but might also help to elucidate some aspects of the structure of the macromolecule.

EXPERIMENTAL

Materials

In this Part, only one extract was employed. It was extracted from the dried *Chondrus* at approximately 80°C., decolorized with carbon at 60°C., and precipitated with ethanol from a slightly saline solution. As shown previously (4) it had a number average molecular weight not greater than 74,500. This extract was chosen on account of its relatively low molecular weight, as it was desired to employ reducing end-group titrations as a means of following the degradation. The material is referred to as extract F.

Solutions were prepared as required by "tumbling" at room temperature until homogeneous, and were clarified by means of Selas No. 02 porcelain filters. The sodium salt of the polysaccharide, which was used in all experiments, was prepared by passing the solutions through a column of Amberlite IR 100 exchange resin. Solutions were stored in the refrigerator at 4°C., but even at this temperature a slight decrease in viscosity occurred on standing. Experi-

[1]*Manuscript received December 6, 1954.*
Contribution from the Maritime Regional Laboratory, National Research Council, Halifax, N.S. Issued as N.R.C. No. 3531.

ments were therefore made as soon as possible after the solutions had been prepared.

Measurements

An iodometric method (11) was employed for the titrations of total reducing power.

Viscosities were measured at 25°C. in Ostwald viscosimeters with the characteristics given previously (4). All viscosities were independent of the rate of shear.

Preliminary experiments showed that the decrease in viscosity which occurred on heating an unbuffered solution of carrageenin was accompanied by a decrease in pH. This catalyzed further degradation so that the reaction became quite rapid in the later stages. Buffered solutions were therefore employed in studying the kinetics. $M/30$ sodium phosphate was chosen since the ionic strength was sufficiently high for adequate buffering capacity but low enough to avoid excessive suppression of the viscosity due to the cation effect.

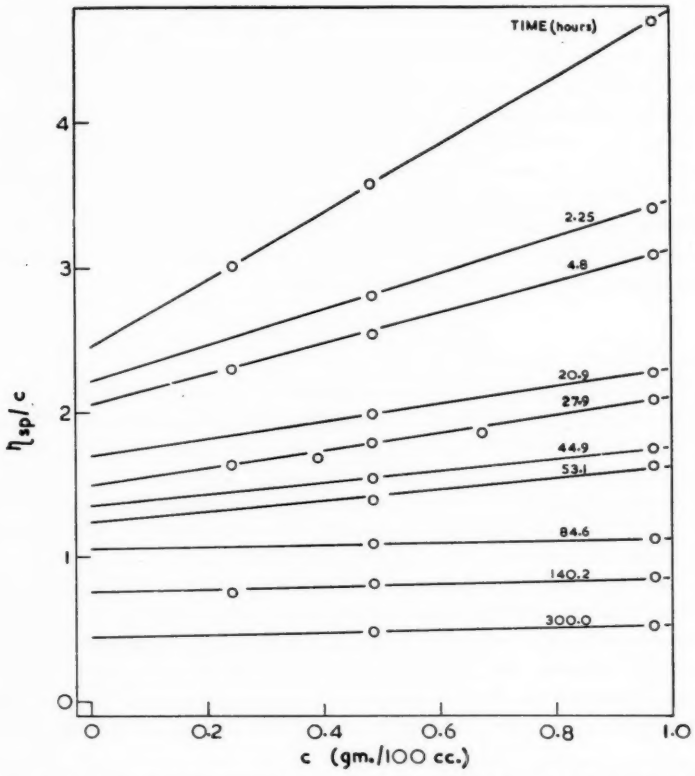

FIG. 1. η_{sp}/c vs. c for a carrageenin solution after various times of heating at 90°C. (Viscosities at 25°C.)

The degradation was studied by heating solutions, of concentration 0.944 gm./100 ml., at various temperatures and measuring the reducing property and intrinsic viscosity of samples withdrawn at various times. The flasks containing the solutions were equipped with reflux condenser, thermometer, and stopper to allow withdrawal of samples, and were placed in baths controlled to within ±0.1°C. Thermal equilibrium was established within one half hour. The experiment at 101°C. was made in an oil bath under gentle reflux and the temperature rose by about 0.5°C. during this experiment. All solutions darkened in color on prolonged heating, although this was slight at 60°C. In addition, small particles of a brown solid occasionally appeared and the solutions were filtered, if necessary, before viscosity measurements were made.

RESULTS

Fig. 1 illustrates the results of a typical experiment. The specific viscosities of the individual samples were measured at various concentrations in $M/30$ phosphate buffer. Extrapolation of the linear plots of η_{sp}/c against c to zero concentration gave values of $[\eta]$ at various times during the experiment. Table I shows the values of $[\eta]$ and of the total reducing power (expressed as

TABLE I

INTRINSIC VISCOSITIES AND END-GROUP TITRATIONS

Time (hr.)	$[\eta]$	mgm. galactose/ 5 ml.	$M \times 10^{-4}$ from equation [1]	Time (hr.)	$[\eta]$	mgm. galactose/ 5 ml.	$M \times 10^{-4}$ from equation [1]
Run 1, Temp. = 90°C.				*Run 2, Temp. = 60°C.*			
0	2.45	0.24	3.926	0	2.29	0.17	3.715
2.25	2.22	0.29	3.614	8.5	2.18	0.20	3.565
4.8	2.06	0.30	3.404	18.8	2.10	—	3.451
20.9	1.70	0.30	2.897	48.0	2.07	—	3.412
27.9	1.50	—	2.612	69.3	1.91	—	3.192
44.9	1.35	0.32	2.388	97.6	2.00	0.25	3.311
53.1	1.24	0.37	2.226	122	1.92	0.33	3.206
84.6	1.05	0.47	1.938	146	1.87	0.34	3.133
140.2	0.75	0.55	1.465	217	1.81	—	3.055
300.0	0.445	1.59	0.948	288	1.78	0.31	3.006
				382	1.74	—	2.958
				478	1.71	—	2.911
Run 3, Temp. = 75°C.				*Run 4, Temp. = 101°C.*			
0	2.29	0.17	3.715	0	2.19	0.23	3.573
3.75	2.10	0.24	3.451	1.95	1.94	0.25	3.236
18.6	1.85	0.29	3.105	5.8	1.79	—	3.027
48.0	1.78	0.32	3.006	11.1	1.56	—	2.698
69.3	1.75	0.24	2.972	20.4	1.32	0.42	2.350
97.6	1.62	0.25	2.786	34.8	0.96	0.46	1.799
122	1.55	—	2.680	45.8	0.84	0.53	1.611
146	1.54	—	2.668	59.3	0.59	0.70	1.199
217	1.41	0.45	2.478	81.8	0.42	0.75	0.904

mgm. galactose/5 ml. of solution) for experiments at different temperatures. Comparison with the titer obtained on complete hydrolysis showed that the degradation was less than 5% in all experiments.

Attempts to measure the molecular weights of some of the samples by their osmotic pressure were unsuccessful as appreciable quantities of low molecular-weight material passed through the cellophane membranes. Molecular weight

TABLE II

COMPARISON OF MOLECULAR WEIGHTS

Polymer	End-group titration (as mgm. galactose/gm. polymer)	Molecular weight from titration	Osmotic molecular weight
FA	3.04	59,000 ± 13,000	42,900
FB	4.92	37,000 ± 6000	30,100
FC	8.85	20,000 ± 1000	21,800

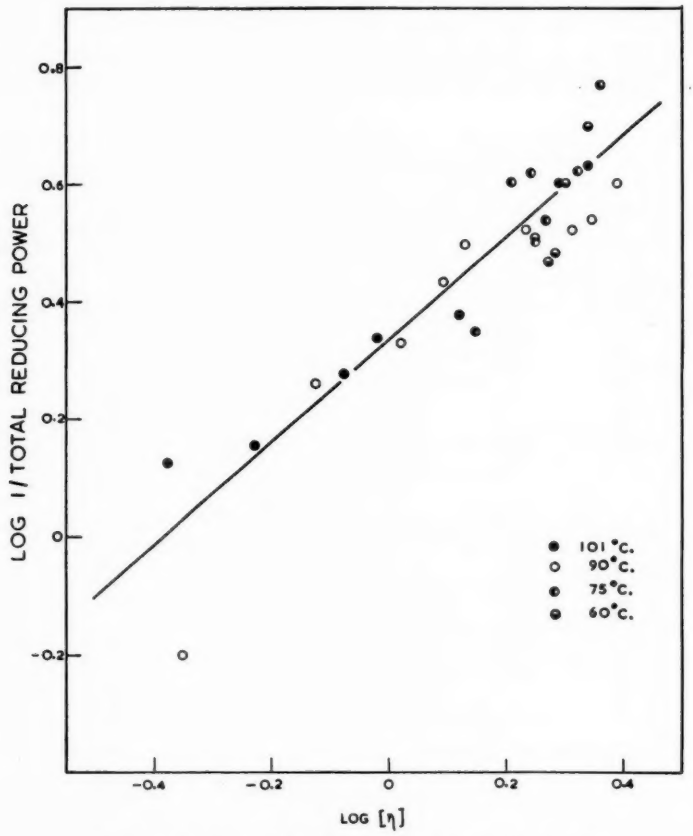

FIG. 2. log [η] vs. log (1/total reducing power) for all runs.

measurements have, however, been performed on dialyzed fractions of the degraded polysaccharide (4). End-group titrations on these fractions showed that the molecular weights calculated on the basis of one reducing end-group per molecule were in reasonable agreement with the accurate values obtained osmometrically. This is illustrated in Table II.

On the basis of this agreement, the titration values shown in Table I have been used to obtain a relationship between $[\eta]$ and M. In Fig. 2 log $[\eta]$ is plotted against log (1/total reducing power) for all the measurements. The scatter is large on account of the insensitivity of the chemical method in this region. For most points, however, the scatter is no greater than expected for an error of ± 0.1 ml. in the titrations. The line through the points corresponds to the relationship

[1] $$[\eta] = 7.59 \times 10^{-6} M^{1.20}.$$

Molecular weights calculated from the intrinsic viscosities by equation [1] are listed in Table I. These values are approximate only, but the relative values may be used to obtain a fairly accurate representation of the course of the degradation.

DISCUSSION

For a first-order, random degradation (2) the velocity constant k is given by the expression

[2] $$1/M_t - 1/M_0 = k\,t/m$$

where M_0 and M_t are the molecular weights at zero time and time t respectively, and m is the molecular weight of the monomer. In Fig. 3, $1/M_t - 1/M_0$ is plotted against t for the four runs. A sharp decrease in molecular weight is observed in the early stages of degradation. After this the plots become linear, indicating random degradation. The initial, non-linear portion of the reaction is exhibited most clearly in the runs at the lower temperatures and does not appear at 101°C. It represents only about 0.3% of the total degradation on the basis of complete hydrolysis.

The slopes of the linear portions of the curves in Fig. 3 give values of k/m. If a tentative value of $m = 250$ is adopted, the following values of k are obtained for the random degradation:

Temp. (°C.)	60	75	90	101
$k \times 10^5$ (hr.$^{-1}$)	0.15	1.13	6.48	20.5

From these data a good Arrhenius plot is obtained, which yields $E = 29.2$ kcal. for the activation energy and $A = 2.75 \times 10^{13}$ (hr.$^{-1}$) for the temperature-independent factor in the equation for the random degradation.

The value of E is of the same order of magnitude as the values found for the degradation of other polysaccharides. Thus Moelwynn-Hughes (8) has reported a value of $E = 30.97$ kcal. for the hydrolysis of maltose polysaccharides. A value of $E = 29$ kcal. has been found (7) for amylose, while for pectin $E = 28 \pm 6$ has been obtained (6).

The values of k are high for a polysaccharide in neutral solution. The labile nature of carrageenin suggests that the observed degradation does not corre-

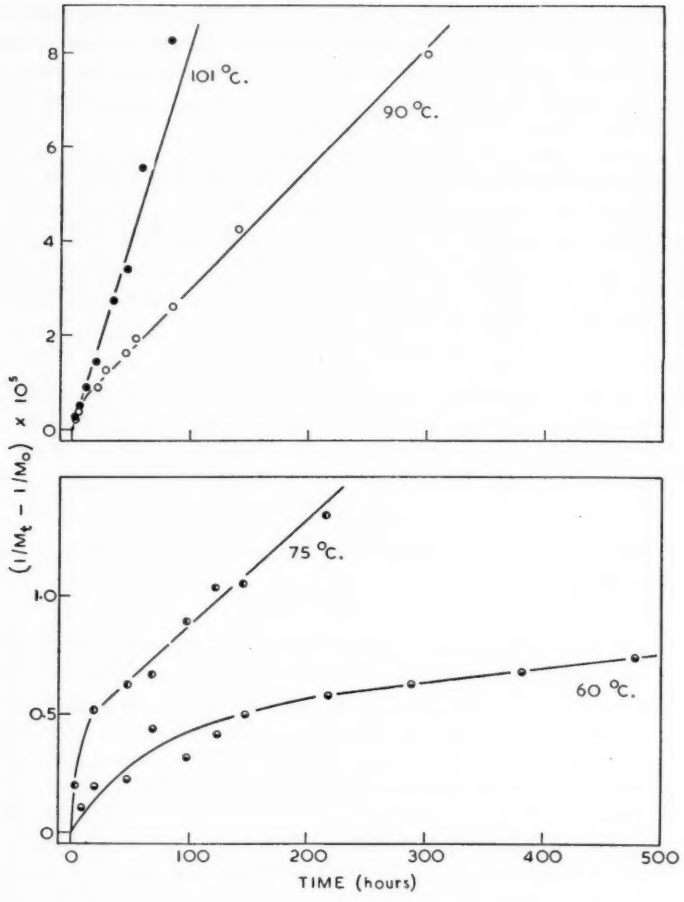

FIG. 3. $1/M_t - 1/M_0$ vs. t for runs at various temperatures.

spond to the scission of 1–3 linked D-galactose residues, but must be attributed to the presence of weaker bonds in the structure. Recent work (9) has revealed the presence of 3,6-anhydro-D-galactose as a constituent of the κ fraction (10) of carrageenin, and strong evidence is available that this compound is split off in the early stages of degradation (5, 9). The high velocity constants may therefore be attributed to this cause.

The random degradation may be interpreted on the basis that the anhydro-D-galactose residues are incorporated either at random or in a regular repeating sequence throughout the structure. The results indicate that long chains of 1–3 linked D-galactopyranose residues are not present in the structure of this extract as the degradation proceeds linearly to low values of $[\eta]$.

At temperatures below 60°C. the rate of random degradation becomes inappreciable. The decrease in viscosity observed for carrageenin solutions at lower temperatures is therefore ascribed to the non-random process noted above. The nature of the weaker linkages responsible for this aspect of the kinetics is at present unknown. A possible interpretation is that bonds responsible for chain-branching or cross-linking are broken in the early stages of degradation, but further work would be required to decide this.

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THE REACTION OF DIETHYL AZODICARBOXYLATE WITH DIHYDROGELSEMINE[1]

By Thelma Habgood and Léo Marion

ABSTRACT

Dihydrogelsemine reacts with diethyl azodicarboxylate yielding a carbinolamine which forms a methyl ether. Both this ether and the carbinolamine base can be oxidized by chromic acid to the same neutral lactam. That there has been no rearrangement of the carbon skeleton during these reactions is shown by reduction of the methyl ether of the carbinolamine with sodium borohydride to dihydrogelsemine and by reduction of the lactam with lithium aluminum hydride to tetrahydrodesoxygelsemine. It is concluded that both dihydrogelsemine and gelsemine contain a methylene group adjacent to $N_{(b)}$, and from the infrared spectrum of the lactam of dihydrogelsemine, $N_{(b)}$ appears to be part of a five-membered ring.

In the course of the investigation of the demethylation of dihydrogelsemine by means of cyanogen bromide (6), the use of diethyl azodicarboxylate as a demethylating agent was explored as an alternate route to dihydro-N-*nor*-gelsemine. It was observed, however, that the action of this reagent on dihydrogelsemine followed a different course. It gave rise to an intermediate which failed to produce formaldehyde when heated with dilute hydrochloric acid, contrary to what was expected from the observations of Diels and Fischer (2) in the preparation of *nor*codeine. The products of the hydrolytic reaction were a new base I and diethyl hydrazodicarboxylate. This seemed to indicate that the azo ester had attacked not the hydrogens of the N-methyl group, but the hydrogen on a carbon adjacent to $N_{(b)}$, and thus afforded a tool with which the alicyclic part of the molecule (5, 7) could be breached.

Because of its unstable character it was not possible to purify the new base I, but on standing in methanol solution in the presence of mild alkali, it was converted into a crystalline methoxy-base II ($C_{21}H_{26}O_3N_2$). The infrared absorption spectrum of this base II (Fig. 1, curve 1) contained two bands (1095 and 1115 cm.$^{-1}$) attributable to ether linkages, together with a band in the NH region and a strong peak at 1713 cm.$^{-1}$ due to the oxindole carbonyl. The base I formed a crystalline perchlorate, $C_{20}H_{23}O_2N_2 \cdot ClO_4$ corresponding to the anhydro-base III, and the infrared absorption spectrum of the salt (Fig. 1, curve 2) indeed showed an absorption band at 1668 cm.$^{-1}$ which has been shown to occur in the spectra of salts containing the $>C{=}N^{+}<$ grouping (3). The methoxy-base II when treated with perchloric acid gave rise to the same anhydro salt as base I. These facts can be interpreted on the assumption that base I is a dihydrogelsemine carbinolamine, and in support of this, the methoxy-base II gives the silver mirror test, reduces Fehling's solution, and within a few minutes gives a dark orange color with 2,4-dinitrophenylhydrazine. This interpretation was confirmed by the reduction of the methoxy base II

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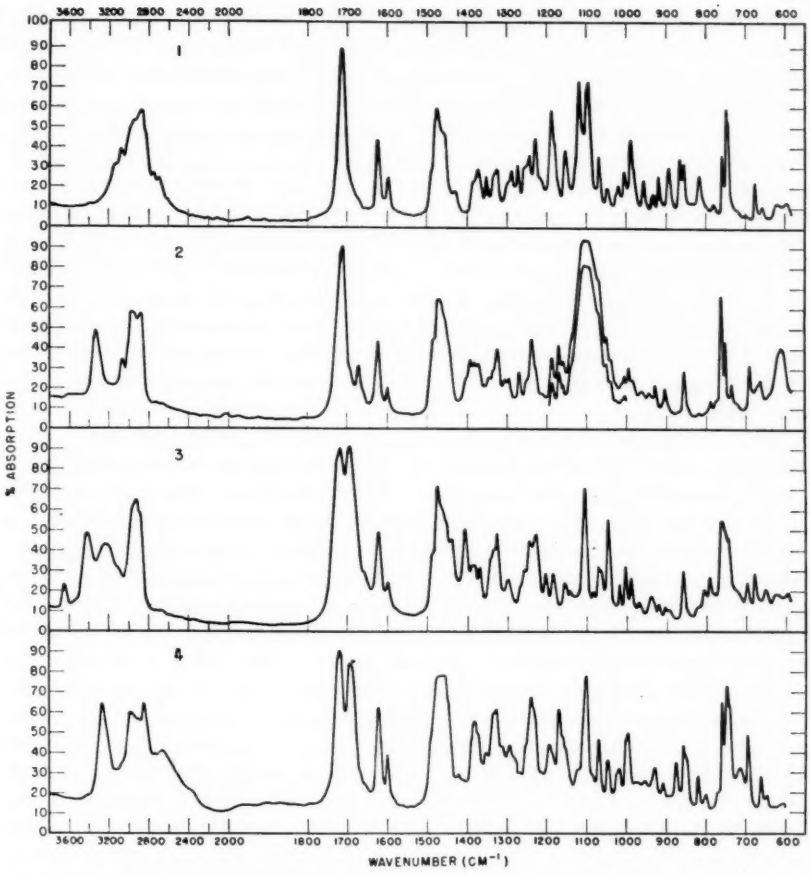
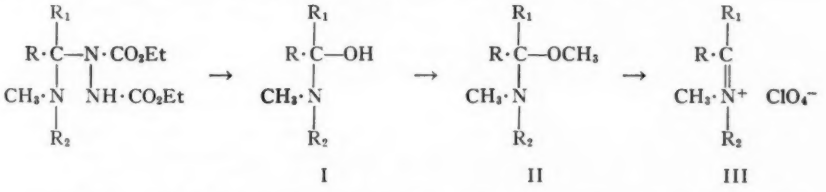


FIG. 1. Infrared absorption spectra in nujol mulls taken on a double beam Perkin-Elmer spectrophotometer, model 21. Curve 1, methoxy base II. Curve 2, perchlorate of base I. Curve 3, dihydrogelsemine lactam. Curve 4, dihydrogelsemine.

with sodium borohydride which converted it to dihydrogelsemine. Hence the above reactions can be represented by the partial formulae I–III.

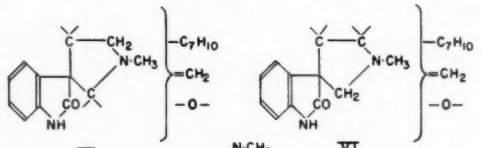


Oxidation of either base I or the methoxy-base II with chromic anhydride in acetic acid gave rise to a neutral product IV ($C_{20}H_{22}O_3N_2$). This product

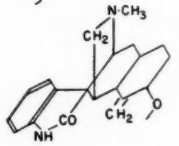
differed from dihydrogelsemine in having two hydrogens less and one oxygen more. Its neutral properties suggested that it was a lactam, and its infrared absorption spectrum (Fig. 1, curve 3) supported this deduction. It showed a strong absorption band at 1718 cm.$^{-1}$ due to the oxindole carbonyl, and another still stronger peak at 1693 cm.$^{-1}$ attributable to a carbonyl present in a cyclic lactam[2]. The absorption of the new carbonyl in the infrared shows it to be part of a five-membered lactam since the carbonyl of six-membered lactams absorbs at lower frequencies. The lactamic nature of the new carbonyl was confirmed by the action of lithium aluminum hydride which converted product IV into tetrahydrodesoxygelsemine, $C_{20}H_{26}ON_2$, identical with that obtainable directly from dihydrogelsemine (7). Product IV, therefore, contains a carbonyl next to $N_{(b)}$ and dihydrogelsemine must contain a methylene group adjacent to the basic nitrogen. The lactam was hydrolyzed by barium hydroxide, but the resulting amino acid recyclized as soon as its aqueous solution was evaporated to dryness.

Since gelsemine is known to contain an exocyclic methylene group (4), and since the conversion of gelsemine to dihydrogelsemine has been shown to involve the saturation of this double bond (4), it is legitimate to assume that this reduction does not cause any alteration of the carbon-nitrogen skeleton. Hence, gelsemine also must contain a methylene group adjacent to $N_{(b)}$. Although the action of diethyl azodicarboxylate on gelsemine did not yield any crystalline substance, the amorphous reaction product was converted back to gelsemine by reduction with sodium borohydride, and this lends support to the foregoing conclusion.

In the light of these results, the structures suggested by Robinson (4) and by Prelog and his co-workers (5) to represent gelsemine are untenable. Of the six rings present in gelsemine the nature of three is now established and the base can be represented by one of the partial structures V or VI of which for biogenetic considerations V is preferred. Since one carbon is required for the exocyclic methylene group and one ring is a cyclic ether, a remnant C_7H_{10} is



V VI



VII

[2]*In this connection it is noteworthy that the infrared spectrum of dihydrogelsemine (in a mull) shows a double peak in the carbonyl region although this base contains only one carbonyl group (Fig. 1, curve 4). The infrared spectrum of N-cyano-*norgelsemine *also contains a double peak in the carbonyl region whereas the spectra of gelsemine and N-cyanodihydro*norgelsemine *show a single absorption band in that region. In the spectrum of the lactam, however, the second peak is stronger than that of the oxindole carbonyl (Fig. 1, curve 3).*

left with which to form the two remaining rings. To represent gelsemine, formula VII is tentatively suggested. It can be derived from one molecule of tryptamine and one of tyrosine which together account for the carbon-nitrogen skeleton of the base. The known reactions of gelsemine can be explained on the basis of this formula in which, however, only one point of attachment of the ether oxygen is suggested, there being no evidence so far as to the size of the cyclic ether.

EXPERIMENTAL[3]

Dihydrogelsemine Carbinolamine

To a solution of dihydrogelsemine (3.643 gm.) in a mixture of acetone (100 ml.) and methanol (10 ml.) diethyl azodicarboxylate (4.0 ml.) (prepared according to the method of Curtius (1)) was added dropwise with swirling. A slight amount of gas was evolved and the solution became warm. After 2.5 hr. the solvent was removed under reduced pressure, and the gummy residue was heated on the steam bath for one hour with 10% hydrochloric acid. No smell of formaldehyde was detected. When cool, the acidic solution was extracted with chloroform to remove neutral compounds consisting of unreacted diethyl azodicarboxylate and of diethyl hydrazodicarboxylate, m.p. 133.2–133.7, either alone or in admixture with an authentic specimen. The acidic solution was alkalized with dilute sodium hydroxide and the precipitated base was extracted with chloroform. On evaporation, the extract left a pale yellow glass difficultly soluble in methanol (3.005 gm.). The basic fraction was benzoylated in chloroform solution with benzoyl chloride (1.6 gm.) under Schotten-Baumann conditions. The chloroform layer was washed with dilute sulphuric acid and the tertiary amine (1.829 gm.) was isolated by alkalization and extraction with chloroform. The tertiary base dissolved in methylene dichloride was chromatographed on alumina. Elution with methylene dichloride and chloroform gave a colorless glass (1.533 gm.). A portion of this base was allowed to stand overnight with methanol, potassium carbonate, and methyl iodide. The mixture was then filtered and the filtrate evaporated to dryness. A residue was left which crystallized from methanol as colorless prisms, softening at 262°, m.p. 264.5–267.5° (decomp.). After two recrystallizations from methanol, the product softened at 258.5° and decomposed at 262.5–264°. It gave no precipitate with silver nitrate solution, was soluble in dilute acids, and could be precipitated from solution by addition of alkali. Calc. for $C_{21}H_{26}O_3N_2$: C, 71.16; H, 7.39; N, 7.91; OCH_3, 8.8; $N\cdot CH_3$, 4.24; one active H, 0.28%. Found: C, 71.17; H, 7.30; N, 7.83; OCH_3, 9.17; $N\cdot CH_3$, 4.28; active H, 0.23%.

Later it was found that the methyl ether could be prepared simply by allowing the tertiary amine fraction to stand overnight in methanol in the presence of potassium carbonate.

The basic methyl ether formed a perchlorate which after two recrystallizations from methanol gave colorless prisms, m.p. 295–295.5°, (dec.) Calc. for $C_{20}H_{23}O_2N_2 \cdot ClO_4$: C, 56.79; H, 5.48; N, 6.63. Found: C, 56.85; H, 5.47; N, 6.72%. It is the perchlorate of the anhydro base and its formation involved

[3] *All melting points are corrected.*

the loss of the elements of methanol. The same salt could be obtained directly from the amorphous unmethylated base.

Reduction of Dihydrogelsemine Carbinolamine Methyl Ether

A solution of the carbinolamine methyl ether (72 mgm.) in methanol (5 ml.) was treated with powdered sodium borohydride (110 mgm.) and allowed to stand for one hour at room temperature. The solvent was evaporated under diminished pressure and the residue was taken up in water and extracted with chloroform. The extract was washed with dilute sulphuric acid, the washings were made alkaline with dilute sodium hydroxide and extracted with chloroform. The base obtained by evaporation of the chloroform (50 mgm.) crystallized on addition of a few drops of acetone, m.p. 219–221°, undepressed by admixture with dihydrogelsemine. The infrared absorption spectrum of the product was exactly superimposable on that of dihydrogelsemine.

Dihydrogelsemine Lactam

The carbinolamine methyl ether (338 mgm.) dissolved in glacial acetic acid (15 ml.) was treated with a solution of chromic anhydride (132 mgm.) in water (1 ml.) and acetic acid (5 ml.). A brown complex was precipitated at first which redissolved. After 18 hr. the excess oxidizing agent was destroyed by addition of a few milliliters of ethanol, and the solution was alkalized with dilute sodium hydroxide. The suspension was extracted with chloroform and the extract washed with dilute sulphuric acid. Evaporation of the chloroform left the neutral lactam (252 mgm.) as a pale yellow foam which was dissolved in benzene and chromatographed on alumina. Elution with ether gave the lactam (186 mgm.) which crystallized as long prisms from methanol. Undried material when heated slowly sintered at 152° and effervesced from 172–180°. When dried at 110° for 10 hr., the lactam had m.p. 157–159.5°. Calc. for $C_{20}H_{22}O_3N_2$: C, 70.98; H, 6.55; N, 8.28. Found: C, 70.70; H, 6.46; N, 8.23%.

Oxidation of Base I

The amorphous base I (338 mgm.) was dissolved in glacial acetic acid (15 ml.) and treated with a solution of chromic anhydride (132 mgm.) in acetic acid (5 ml.) and water (1 ml.). After 18 hr. at room temperature a little ethanol was added to destroy the excess of chromic acid, the solution was diluted with water, alkalized with sodium hydroxide, and extracted with chloroform. The chloroform extract was washed with dilute sulphuric acid and with water, and evaporated to dryness. The neutral product (252 mgm.) was dissolved in benzene and chromatographed on alumina (activity IV) and eluted with ether. A crystalline fraction (116 mgm.) was obtained which after recrystallization from methanol consisted of colorless prisms. After drying it melted at 157–159.5° either alone or in admixture with the dihydrogelsemine lactam obtained by oxidation of the carbinolamine methyl ether.

Reduction of Dihydrogelsemine Lactam

A solution of dihydrogelsemine lactam (260 mgm.) in dry dioxane (12 ml.) was added dropwise to a suspension of lithium aluminum hydride (650 mgm.) in freshly distilled ether (1 ml.). After the vigorous reaction had abated, the

mixture was refluxed for two hours and afterwards the excess reagent was destroyed by the cautious addition of 1:1 methanol–water. The precipitated hydroxides were centrifuged and washed with methanol, the decantate and washings were evaporated under diminished pressure, and the residue dissolved in chloroform. Evaporation of the filtered chloroform solution left a yellow gum (414 mgm.), the benzene-soluble portion of which was chromatographed on alumina. Elution with benzene yielded a colorless oil (190 mgm.) which crystallized from ether as colorless flat prisms, m.p. 142–142.5°, undepressed on admixture with an authentic specimen of tetrahydrodesoxygelsemine (7).

Saponification of the Lactam

Refluxing dihydrogelsemine lactam with potassium hydroxide in ethanol, or with aqueous dilute hydrochloric acid or concentrated hydrochloric acid did not hydrolyze the lactam. Heating with concentrated hydrochloric acid in a sealed tube at 160–180° also failed to bring about hydrolysis.

The lactam (375 mgm.) was heated in a sealed tube for five hours at 150–160° with saturated aqueous barium hydroxide solution (10 ml.). After cooling, the solution was filtered and to the filtrate small pieces of dry ice were added to precipitate the barium. The barium carbonate was centrifuged and washed with hot water. Evaporation of the combined decantate and washings almost to dryness gave 315 mgm. of long platelets which became brown above 200° but did not melt below 310°. Evaporation to complete dryness transformed the solid into a chloroform-soluble substance (185 mgm.) which crystallized from methanol as prisms softening at 155–168°, shown by its infrared absorption spectrum to be identical with the original lactam. The lactam was inert to 95% hydrazine at 125–135°.

Reaction of Gelsemine with Diethyl Azodicarboxylate

Gelsemine (2.569 gm.) in methanol solution was allowed to stand for 18 hr. with diethyl azodicarboxylate (1.754 gm.), and the product worked up exactly as described for dihydrogelsemine. It consisted of a colorless glass which did not crystallize from any of the usual solvents.

A quantity of the product (103 mgm.) dissolved in methanol (15 ml.) was treated with sodium borohydride (302 mgm.) exactly as described above. The product after chromatography on alumina consisted of a colorless foam (34 mgm.) which crystallized from acetone, m.p. 178.5–179°, undepressed on admixture with gelsemine.

ACKNOWLEDGMENT

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DISINTEGRATION-RATE DETERMINATION BY 4π-COUNTING
PART I[1]

By B. D. Pate[2] and L. Yaffe

ABSTRACT

The response probability of a 4π-counter has been examined and conditions determined for which the response probability is unity to within $\pm 0.1\%$ for a range of nuclides. Variables examined have been the effect of polarization potential, discriminator bias, source location, counting rate, counter gas purity, thickness of conductor on source mount, for particles of various energies and from various modes of disintegration.

GENERAL INTRODUCTION

The determination of absolute disintegration rates in a precise manner is of fundamental importance in nuclear physics and nuclear chemistry. It is also of some application in other fields, e.g. radiobiology, where radioactive tracers are used as a research tool. The experimental methods available have been reviewed a number of times (8, 18, 25, 26). All with the exception of 4π-counting and coincidence counting are subject to sources of error which limit the accuracy attainable to several per cent. Recent results (31) with β–γ and γ–γ coincidence measurements indicate that an accuracy of better than one per cent may be possible. The method, however, is limited to nuclides which emit gamma radiation and whose decay schemes are well known.

4π-Counting, up to this time used mainly in the standardization of material for therapeutic use, has considerably wider applicability and greater potential precision than the majority of other techniques. However, the results of international intercomparison of standards (31) show a scatter of a few per cent, and the limits of error assumed by individual workers are of the same order of magnitude (16, 18, 26, 31, 33).

Errors of this magnitude are certainly not inherent in the method and the present paper is the first of a series which will describe work aimed at improving the accuracy attainable with 4π-counting techniques. While some of the information we shall present is strictly applicable only to the design of chamber we have used, most of it is of interest in connection with any form of 4π gas-ion counting.

4π-Counting in the present form originated in 1944, when Simpson (32) described a counter with a geometry approaching 4π steradians. What was probably the first true 4π-counter was designed by L. Meyer-Schützmeister and described in 1948 by Haxel and Houtermans (17). Since that time a number of counting chambers of a variety of designs have been described (1, 2, 4–7, 16–18, 22, 23, 27, 28, 30, 32, 33, 35–37). A counter system with a geometry of 4π steradians can be achieved in several ways, e.g. with a gaseous source as part of the filling of a gas counter, or with the source dispersed

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throughout the phosphor of a scintillation counter. We have confined our attention to a system which has obvious advantages for routine radiochemical work—a chamber composed of two 2π gas-ion counters arranged face to face. The source material is placed between them, in solid form.

The particular advantages of a counter that will respond to radiation emitted over a solid angle of 4π steradians have been appreciated for some years. Since every charged particle that is emitted is registered by the counter system, any event subsequent to charged particle emission which occurs within the resolution time of the instrument will not be registered separately. This has two desirable consequences.

(i) Provided the primary events of the nuclear transition studied include charged particle emission with a total probability of unity, one count will be registered per distintegration. This remains undisturbed by the emission of secondary particles, nuclear gamma radiation, or annihilation radiation, when this occurs within the counter resolving time, as is usually the case. Cases for which some data from the decay scheme are required include those when particle emission occurs with a probability of less than unity (which includes orbital electron capture) and transitions leading to a metastable excited state of the product nucleus. In such cases, the accuracy of the disintegration-rate determination will clearly depend on the accuracy of the nuclear data employed, in addition to that inherent in the counting procedure.

(ii) Scattering of radiation either by source material, source mount, gas, or counter walls is without effect on the observed disintegration rate, since any discharges (subsequent to that corresponding to the primary event) caused by repeated scattering of one particle will occur within the counter resolving time. The same is true of secondary radiation production due to interaction of primary particles with gas, wall, or other material within the counter.

Thus most of the large corrections (38) of uncertain accuracy required to correct data from conventional counter systems (where the geometry is much less than 2π steradians) are eliminated. The only sources of error still to be corrected for in the calculation of disintegration rates from counter data are due to:

(*a*) Failure of the counter to respond once to every ionizing particle, arising from a nuclear disintegration, that reaches the counter gas.

(*b*) Absorption of radiation by the mount on which the source is deposited.

(*c*) Absorption of radiation by the source material itself ('self-absorption').

(*d*) Statistical fluctuations in the disintegration rate of the source and in the background counting rate of the counter. This error can clearly be reduced to within any desired limits by extension of the number of events observed.

This paper considers the first of the above items. Subsequent publications will deal with (*b*) and (*c*) and certain theoretical topics relating to counter behavior.

INTRODUCTION

For the present discussion, it is convenient to define a quantity which we shall call the ***response probability*** of the counter. This is the probability that a

discharge triggering the recorder be produced when a charged particle or photon originating from a nuclear disintegration reaches the counter gas. (The term "efficiency" is avoided since it has been used by different workers in various contexts.) This probability will have a certain value for a specific form of radiation (e.g. for charged particles ideally it will be unity) but under given conditions however it may deviate from this value. It is the purpose of the remainder of this paper to describe an examination of the variation of the response probability of the counter system used by us.

The response probability is found to be a function of several variables in the conditions under which the counter is operated. Some of these variables can be adjusted to ensure that no deleterious effect on the response probability occurs. Others cannot be controlled in this fashion and one must determine, to the desired degree of precision, the value of response probability as a function of the variable considered. Corrections for the departure from unit probability in the final calculation of disintegration rate can then be made.

The response probability of the counter may be less than unity if:

(*a*) the gas multiplication of the chamber is less than a minimum value;

(*b*) the "effective geometry" of the chamber is less than 4π steradians;

(*c*) the counting rate is so high that the apparatus is unable to resolve successive discharges, and "coincidence-losses" occur.

It may be more than unity, i.e. more than one count be registered per disintegration, when:

(*d*) at higher anode voltages, the discharge phenomena become more energetic. Then photon emission from the excited gas molecules and positive ion bombardment producing secondary and photoelectron emission from the cathode may lead to multiple discharging over a time interval greater than the counter resolution time.

(*e*) the counter gas contains more than a limiting concentration of impurities with an electron affinity. This may lead to the formation of slow moving negative ions which, collected after the electron pulse, may also lead to spurious pulses being observed.

(*a*) *Gas Multiplication*

In order that the passage of an ionizing entity through the chamber shall register a count, the ionization produced, after gas multiplication, collection at the counter anodes, and external amplification, must produce a pulse at the output of the amplifier system larger than the discriminator bias potential applied to the signal. Whether this condition is satisfied depends on the value of the multiplication factor, amplifier gain, capacitance of the chamber anode system and amplifier input, and the bias potential. These quantities may all be measured, but, aside from the discriminator bias, which cannot be reduced below a minimum value, the only one normally varied in the system is the gas-multiplication factor. This must be adjusted, by increasing the polarization potential for example, until the situation described above is obtained. However, the polarization potential cannot be raised indefinitely since above a limiting value the secondary processes described under (*d*) above set in.

Thus it is important to determine whether the gas multiplication can be raised sufficiently to bring the response probability of the chamber close to a value of unity for the radiation producing the least amount of ionization in the chamber. (This is clearly particularly important when beta radiation is to be examined, since the energy spectrum includes a proportion of particles with very low energies, a proportion which increases with decreasing spectrum energy end point.)

One may determine in two ways whether this state of affairs can be brought about.

(i) The change in counting rate as the gas multiplication is varied within the range available may be observed.

(ii) The various quantities described above, including the gas multiplication (as a function of polarization potential), may be measured and the total response probability of the counter under given conditions may be calculated for those energies where specific ionization is at a minimum.

Both these methods have been used in the present investigation.

In addition to being a function of the polarization potential, the gas multiplication is also dependent on several other variables.

(iii) The polarization field strength is a function of position inside the chamber, and therefore the ion collection will be a function of source position both within the normal source-mount plane, and for movements at right angles to this plane. However, at certain polarization potentials, it is expected that a region will exist in the center of the chamber over which the response probability is constant at a value of unity. The operation of the counter will then be insensitive to small movements of the source within this region.

(iv) Since the efficiency of ion collection within the chamber is a rapidly varying function of field strength it is important to ensure that in the vicinity of the source the field strength is at a maximum. This is especially true for soft β emitters which will have a short range in the gas and thus most of the ionization is produced in the immediate source vicinity. The counter design ensures this maximum field strength provided the source mount is conducting to the cathode surface. An insulating mount will disturb the field strength in such a manner that a position of zero field strength occurs at the source position and hence poor ion collection results.

Seliger and Cavallo (30) claimed that the loss in efficiency due to the use of a non-conducting film mounted over an aperture in an aluminum diaphragm was negligible provided the aperture diameter was limited to minimize the field disturbance. However, a later paper (22) agreed with most observers that, for reliable results, the film had to be rendered conducting. This is satisfactorily accomplished by distillation of gold *in vacuo* on to the film. A rapid routine method for estimating the thickness of the gold layer has been described in a previous publication (24). It is of considerable importance to reduce the superficial density of the source mount used as far as possible in order to reduce absorption of radiation (see the next paper in this series). It is thus essential to know the minimum superficial density of gold required for satisfactory counter operation, since it is fruitless to reduce the film super-

ficial density much below this figure. Little quantitative information is available on this point, or on whether one or both sides of the film need be coated. Hawkings (16) has used 25 μgm./cm.^{2} of gold on both sides of a 100 μgm./cm.^{2} Formvar film with success.

(v) The electron-collection process will clearly be affected to some extent if the counter gas contains a significant proportion of electron-accepting impurities. It is therefore of importance to determine the limiting concentration which can be tolerated without the counter operation being affected.

(*b*) *Counter "Geometry"*

The response probability will be reduced from a value of unity if conditions are such that a proportion of the emitted radiation is absorbed (other than by source material, or the mounting film) before it can produce the necessary minimum number of ions required to trigger the recorder. It must, therefore, be verified that, with the aperture sizes used by us, this effect is not serious.

(*c*) *Coincidence Losses*

Operation of the chamber as a proportional counter serves to increase the value of the response probability at high counting rates, by making possible a reduction in the resolution time of the apparatus, compared with that obtained with the counter operated as a Geiger-Müller counter for instance. The use of the 4π-counter over a wide range of counting rates nevertheless demands that the coincidence loss be accurately known as a function of counting rate. Owing to the variety of processes which may cause coincidence losses in the proportional counter and associated electronic equipment this function is best obtained empirically. The availability of thin films, described in a previous publication (24), has made possible a very precise determination of the coincidence loss function by a modification of the conventional multiple source technique.

EXPERIMENTAL PROCEDURES, RESULTS, AND DISCUSSION

The counter chamber used in these experiments is essentially that described by Hawkings *et al.* (16) and is reproduced in Fig. 1. It consists basically of two hemispherical brass cathodes of 7 cm. diameter and two ring-shaped anodes of 0.001 in. diameter tungsten wire. The source mount, which is continuous with the cathodic surface, consists of a plastic film of superficial density 5–10 μgm./cm.^{2} rendered conducting by a layer of gold (2 μgm./cm.^{2}) applied by distillation *in vacuo* to either surface of the film. The film is mounted over an aperture in an aluminum plate 1 mm. thick. The aperture diameter is usually 5 cm. and occasionally 2.5 cm.

The chamber is used in conjunction with a Nicholls high voltage supply (AEP 1007B). The anodes of the chamber are connected in parallel to an Atomic Instrument Co. preamplifier (205-B) which has been modified to obtain a low noise level. The output from this is fed into an Atomic Energy of Canada Ltd. amplifier (AEP 1448) which has non-overload characteristics, and thence after discrimination into a Marconi scaling unit of 1000 (AEP 908).

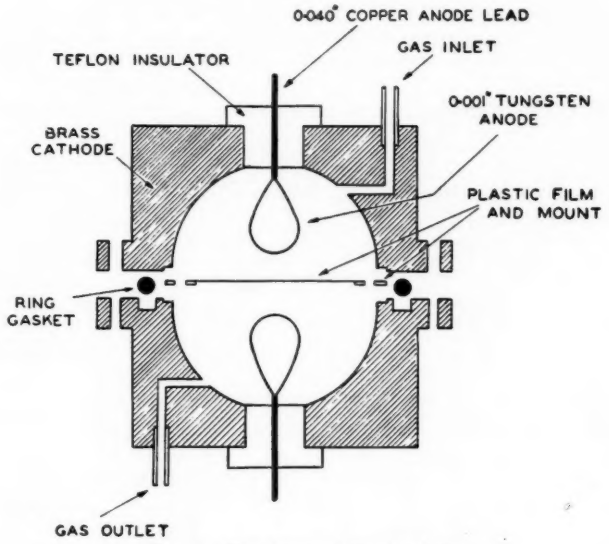


FIG. 1. The 4π-counting chamber.

The over-all gain of the amplifying system is 30,000 and a bias voltage up to 50 v. can be applied by the discriminator to the output signal.

The counter chamber is operated in the proportional region. The counting gas used is C.P. methane at atmospheric pressure dried by passage over silica gel. After insertion of the sample the counter is flushed for about five minutes at a rapid flow rate and the rate then moderated to a standard rate of 0.5 ml. methane per sec.

The stability of the equipment, which was checked with a standard RaD-E-F source and a Ni^{63} source, is excellent. This can also be attested to by the reproducibility of results obtained at different times.

The sources of radioactivity used were all of so high specific activity that source thickness did not affect the results quoted in this paper. Samples approximating "point-sources" were prepared by evaporation of a suitably sized aliquot of stock solution on the mounting film using infrared radiation. One exception to this procedure was the case of Tc^{99m}. This nuclide was co-separated with rhenium in the normal fission product procedure (14) up to the final stage when the Re–Tc mixture was precipitated as the sulphide. The sulphide was slurried with water and a portion evaporated on the film.

The half life, mode of decay, radiation characteristics, and source of supply of the nuclides used are shown in Table I.

(*a*) *Gas Multiplication*

(i) *High Voltage Characteristics of the Counter*

High voltage characteristic curves were obtained in the usual way for a series of sources of β radiation of increasing maximum energy, at a constant

TABLE I

CHARACTERISTICS AND SOURCE OF SUPPLY OF RADIOACTIVE NUCLIDES

Nuclide	Half life	Mode of decay	Maximum energy of particle (Mev.)	Source of supply
Ni^{63}	80 yr.	β^-, no γ	0.067	A.E.C.L.
S^{35}	87 days	β^-, no γ	0.167	A.E.C.L.
Ca^{45}	152 days	β^-, no γ	0.254	A.E.C.L.
Tl^{204}	4.0 yr.	β^-, no γ	0.765	A.E.C.L.
Bi^{210}	5.0 days	β^-, no γ	1.17	Separated from RaD-E-F from A.E.C.L.
P^{32}	14.4 days	β^-, no γ	1.70	A.E.C.L.
La^{140}	40.3 hr.	β^-, γ	2.26	Separated from fission products of uranium irradiated in BEPO, A.E.R.E. Harwell, England
Po^{210}	138 days	α	5.30 (mono-energetic)	Separated from RaD-E-F from A.E.C.L.
Na^{22}	2.6 yr.	β^+, γ	1.8	A.E.C.L.
Co^{60}	5.3 yr.	β^-, γ	0.319	A.E.C.L.
Tc^{99m}	6.0 hr.	IT	0.142 (mono-energetic)	Separated from fission products of uranium irradiated in NRX, Chalk River, Ont.

discriminator bias level. The nuclides employed, with the exception of La^{140} all emit β^- particles without accompanying γ radiation. Their nuclear properties are described in the top half of Table I. Fig. 2 indicates the response of the counter to pure β radiation. Standard deviations on the quantities measured lie within the areas of the points as plotted (as for all succeeding figures in this paper unless otherwise indicated).

The data shown in Fig. 2 indicate that the chamber used in this work is suitable for measurements of β^- emitters with maximum energies at least down to 0.067 Mev. The plateaus obtained in no case show any perceptible slope. The maximum figure which can be calculated on a basis of the statistics of the measurements concerned is less than 0.1% per 100 v. There is, therefore, little ambiguity as to the correct counting rate figure to be used as a basis for calculating the disintegration rate, and little doubt of the fact that substantially all of the particles in the spectrum are being registered. At polarization potentials of about 100 v. in excess of the threshold value, the response probability approximates very closely to unity. However, obviously, any polarization potential on the plateau may equally well be used if for any reason this is desirable.

The displacement observed with increasing particle energy is due to the reduced amount of ionization produced in the region of maximum polarization field strength by the more energetic radiation. The effect is less marked at the

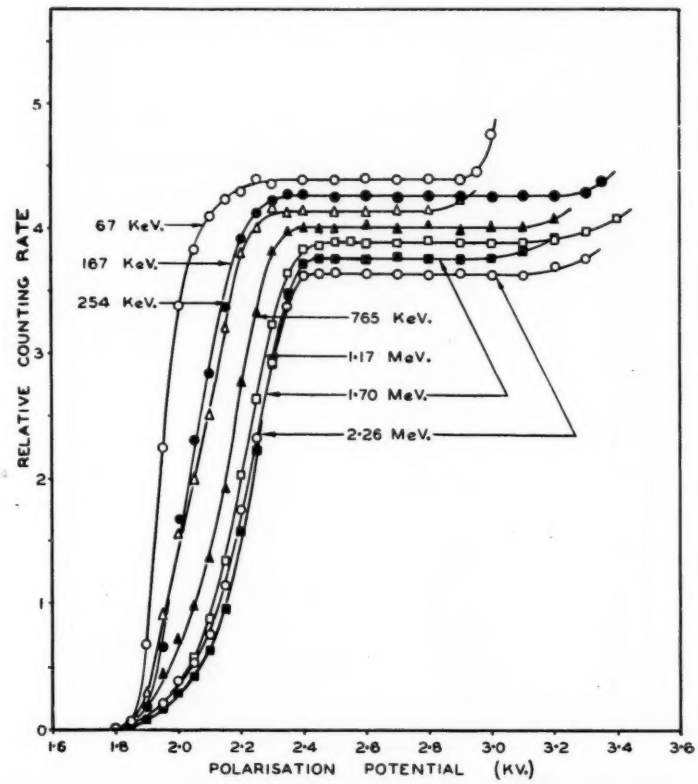


FIG. 2. High voltage characteristics for β radiation of varying maximum energies.

higher end of the energy scale since there is very little variation of specific ionization witn energy in this region.

The length of the plateaus observed with β^- emitters is never less than 400 v. and may be as much as twice this. The polarization potential at which multiple pulsing sets in is dependent on many factors such as condition of source and temperature of the chamber.

Fig. 3 shows a series of characteristics obtained using Ni^{63} (maximum energy 0.067 Mev.) taken at increasing discriminator bias values. Fig. 4 shows a similar series using La^{140} (maximum energy 2.26 Mev.). Plateaus taken at increasing bias values are displaced to higher polarization potential values but otherwise are exactly superimposable. Fig. 5 shows characteristics obtained with nuclides decaying by the emission of alpha particles, positrons, negative electrons with gamma radiation, and by isomeric transition. These data indicate that the present chamber makes a satisfactory counter for many forms of radiation. Absolute measurements with α- and β^+- and β^--emitting nuclides with or without accompanying γ radiation are clearly straightforward.

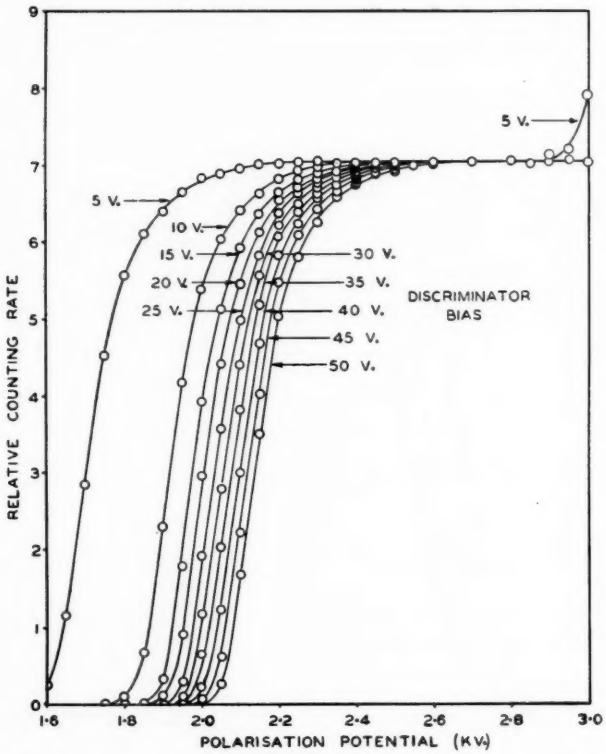


FIG. 3. High voltage characteristics for Ni^{63} β radiation as a function of applied discriminator bias.

With nuclides decaying by means of isomeric transition the same is true if the pertinent nuclear data are available.

Fig. 6 shows the variation of the characteristic with increasing disintegration rate. A series of P^{32} sources of known strength was prepared on 5–10 μgm./cm.^{2} films. The disintegration rates (about 500 d.p.s.) were below the region where coincidence losses were significant. A series of secondary sources was then prepared from these by lamination, the total disintegration rates being calculated by summing those of the component sources. (It will be shown in a forthcoming publication that the absorption of radiation from P^{32} even with 10 or more of these component films can be neglected.) The results of measurements at two values of discriminator bias are shown. The calculated rate for each source used is shown for comparison purposes.

These results show clearly that satisfactory characteristics are retained with our apparatus at least up to a counting rate of 3×10^3 counts per second. The irregular shape of the curves above this figure show that it is meaningless to quote a coincidence loss for a counting arrangement unless this loss is given

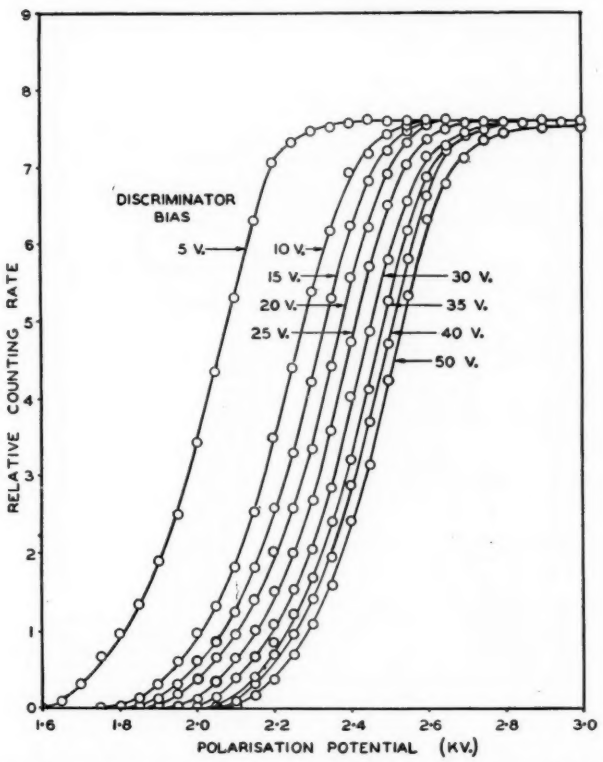


FIG. 4. High voltage characteristics for La^{140} β radiation as a function of applied discriminator bias.

for definite polarization conditions, since coincidence losses in proportional counting are due to a variety of reasons. For example, since the discharge mechanism results in a local and partial reduction of the polarizing field, the counter may enter into successive discharge processes with the effective counter field at different stages of recovery. Also, at higher counting rates, the slow migration of positive ions to the cathode may substantially reduce the polarization field and thus the gas multiplication. In addition to this losses in the electronic circuit may also occur.

(ii) *Response Probability to Charged Particles as a Function of Their Energy*

The ionization produced by most charged particles is more than adequate to ensure a response probability of unity under normal operating conditions. With low energy particles and electrons of about 2 Mev. (where the specific ionization is at a minimum (3)) this may not be so. For example, a small low energy fraction of any spectrum of β radiation will not count with unit probability. This fraction increases as the maximum energy of the spectrum decreases.

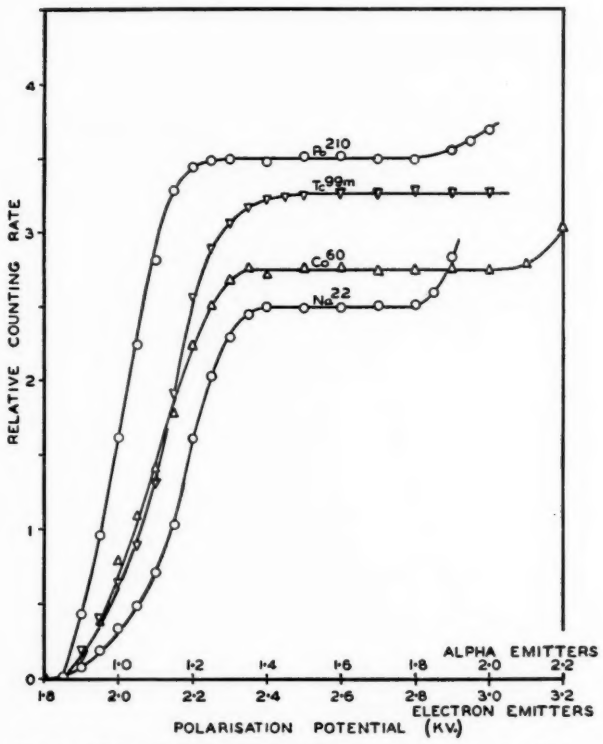


FIG. 5. High voltage characteristics for various forms of radiation.

We have calculated the response probabilities of the counter towards the entire spectrum of the β radiation of Ni^{63} and S^{35} since these were the softest β radiations studied. The calculations are conveniently made in three stages. *First*, the minimum total charge pulse arriving at the counter anode system which will trigger the recording mechanism is calculated from the measured electronic parameters. The corresponding original ionization is then computed.

Amplifier gain	$= 3 \times 10^4$	(measured with a calibrated pulse generator and oscilloscope)
Minimum bias level	= 3 v.	(i.e. a pulse greater than this will trigger the scaler circuit)
Minimum input	= 0.1 mv.	(calculated from above)
Capacitance of chamber anode system and amplifier input	= 25 $\mu\mu$f.	(measured by resonance bridge method)

Therefore, the 0.1 mv. input signal corresponds to a charge arriving at the anode of 2.5×10^{-15} coulombs or 1.6×10^4 electronic charges.

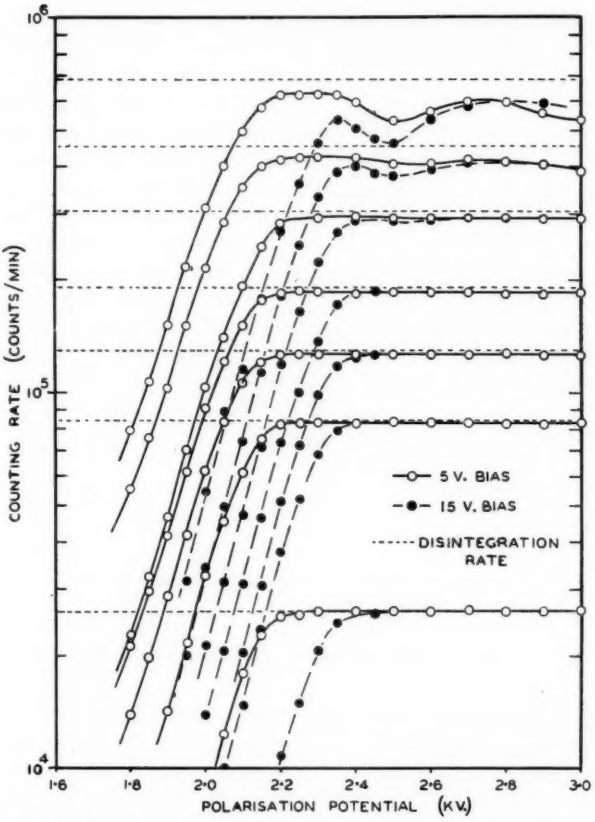


FIG. 6. High voltage characteristics as a function of disintegration rate.

The mean gas multiplication was determined in the following way. Using the alpha particles from Po^{210} the maximum pulse height obtained at the chamber anode system was measured with a cathode ray oscilloscope. Care was taken that pulses were not large enough to produce amplifier saturation or proportional counter distortion. The observed pulse amplitude was independent of polarization potential between 250 and 500 v. Here the chamber is acting as a saturated ion chamber and the gas multiplication is unity. Readings, taken at increasing potentials, were normalized to this value. When the danger of saturation or distortion became apparent a Ni^{63} source was substituted for the Po^{210}. A large range existed where both could be used so that no errors were introduced by the substitution.

The results are shown in Fig. 7. At 2.6 kv. the mean gas multiplication (A) has a value of 2.6×10^3 and at 2.9 kv., $A = 1.6 \times 10^4$. Therefore, at these polarization potentials, the counter will respond to a mean of six and one ion pairs respectively.

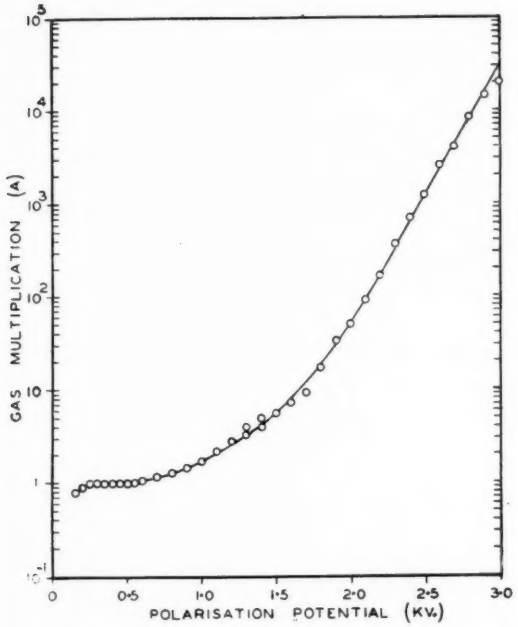


FIG. 7. Gas multiplication function of counter.

Secondly, the probability that a given particle energy will produce a certain minimum number of ion pairs should be calculable if the appropriate data are available. Curran *et al.* (9) note that the mean energy (W) necessary to produce one ion pair increases with particle energy at low energies, but give no quantitative data. They also note that W for both argon and nitrogen (both non-electron acceptors like methane) is substantially independent of energy down to 500 ev. This confirms theoretical predictions made by Fano (11). As a first approximation (adequate for present purposes) one can assume that the value due to Schmeider (29) of $W \sim 30$ ev. applies in the region of interest.

Both the ionization and gas multiplication obtained under given conditions are subject to variance. Let J_0 be the mean number of ion pairs produced in the chamber ($= E/W$ where E is the particle energy dissipated in the gas). The variance of J and of the gas multiplication A have been studied by a number of workers. Fano (12) has shown, on theoretical grounds, that the variance of J, $V_J = FJ_0$ where F (a constant for the particular gas) is found to be equal to 0.3 for atomic H where necessary data for calculation are available.

The variance of A was examined theoretically by Snyder (34) and Frisch (13) who found that $V_A = A$. Thus the total variance V of the output pulse amplitude of a counter expressed as an equivalent variance of the original ionization J will be $V = FJ_0 + J_0$. The observations of Hanna *et al.* (15) at

250 ev., 2.8 kev., and 17.4 kev. indicate that a value of $V = J_0$ describes the experimental results more exactly. Since F is not expected to be very small, the variance of the gas multiplication factor must be less than J_0, confirming the observations of Curran *et al.* (10).

Thus the distribution of pulse amplitudes at the chamber anode for a given energy will be approximately a Poisson distribution. On this basis, then, one can calculate the probability P that at least one or at least six ion pairs be produced, as a function of particle energy, corresponding to the polarization potential values mentioned above.

The probability functions are shown in Fig. 8. Since a minimum of one ion pair will trigger the counter at 2.9 kv., the curve for $J = 1$ represents the counter response probability function under this condition. Similarly, the curve for $J = 6$ gives the response probability at 2.6 kv. Clearly, at low E values these calculations are in error, but certainly show at worst an order of magnitude for P at these energies.

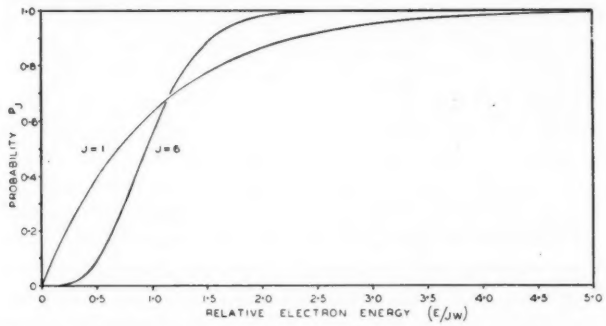


FIG. 8. Counter response probability as a function of electron energy for mean ionizations of one and six ion pairs.

Thirdly, from the probability curves of Fig. 8 it is now possible to obtain a mean response probability for a radiation of any known energy distribution. We have calculated the values for Ni^{63} and S^{35} at applied potentials of 2.6 and 2.9 kv. The relevant probability function has been integrated using energy values from the spectrometer data of Langer *et al.* (21) for S^{35} and Kobayashi *et al.* (19) together with the modified beta decay theory for $\Delta J = 2$, yes transitions (20) in the case of Ni^{63}. The results of the calculations are shown in Table II.

TABLE II

COUNTER RESPONSE PROBABILITY

Nuclide	Applied voltage (kv.)	Mean no. of ion pairs detected	Probability
Ni^{63}	2.6	6	0.9953
Ni^{63}	2.9	1	0.9992
S^{35}	2.6	6	0.9998
S^{35}	2.9	1	0.9999

Owing to their low energy emission Ni^{63} and S^{35} are the two nuclides we have studied which are most likely to have a low response probability. The response probability in the two cases is seen to be very close to unity. This conclusion is also, of course, corroborated by the observations recorded in Fig. 2. The counting rates obtained with all energies remained essentially unchanged even when for example, in the case of S^{35}, the applied potential was increased from 2.4 to 3.2 kv., corresponding to a change in the gas multiplication by a factor of about 160. A minimum in the specific ionization also occurs at a particle energy of about 2 Mev. The specific ionization at this point is about 40 ion pairs per cm. per atmosphere of methane (3) which corresponds to a production of at least 100 ion pairs in the chamber. This is clearly sufficient to ensure a response probability of unity.

(iii) *Response Probability as a Function of Source Position*

We have examined the variation of response probability of the counter for sources of radiation as a function of their location in the source-mount plane in a manner essentially similar to that employed by Hawkings *et al.* (16) for P^{32}. We have determined this for β emitters of varying energy from 0.067 Mev. to 2.26 Mev. at various polarization potentials and bias voltages.

The source was mounted on a small area of 5–10 μgm./cm.^{2} film supported over an aperture of 5 mm. diameter in a slide of aluminum foil. The slide was arranged to move between two parallel wires so that the source could be adjusted to any position along a diameter of the source-mounting diaphragm. This movement, combined with the rotation of the diaphragm, allowed the source to be brought to any point within the plane of the source mount. The whole was screened electrostatically from the electrode system by being sandwiched between two gold-coated 10 μgm./cm.^{2} films. Attenuation of the β radiation by absorption in the small layer of gas trapped between the films could be neglected for purposes of this experiment. The counting rate was observed with the source in 90 different positions, each at two values of polarization potential and two discriminator bias settings. Typical contours of qual response probability are shown in Figs. 9 and 10.

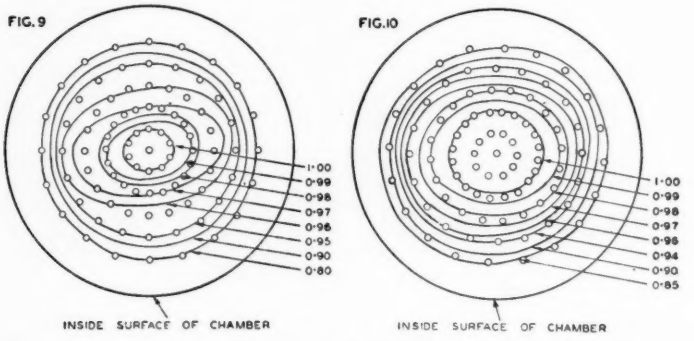


FIG. 9. Contours of equal response probability for Ni^{63} β radiation.
FIG. 10. Contours of equal response probability for La^{140} β radiation.

The contours are essentially similar to those obtained by Hawkings *et al.* (16) for P^{32}. The area over which the response probability is unity for a particular applied voltage and bias level can be seen to be smaller for Ni^{63} than for La^{140}, because of the reduced range of the weaker radiation in the counter gas. Other curves, similar in nature, have been obtained with varying polarization and bias level but are not shown here for reasons of brevity. These curves show an expansion of the contours with increasing polarization and decreasing bias voltage.

From these data it is clear that a source diameter of up to at least 1 cm. can be tolerated by the counter for all particle energies studied without a significant drop in response probability.

It will be seen from Fig. 1 that the counting chamber is constructed in such a manner that both hemispheres are recessed to accommodate an aluminum source-mount diaphragm. This allowed us to investigate the variation of response probability when a source of S^{35} was moved 1 mm. from the medial position towards each anode. (The source-mounting film is customarily affixed to one side of the aluminum diaphragm, and the source material deposited on the side of the film away from the diaphragm.) The results, as shown in Table III, show that a displacement of at least 1 mm. in either direction (the maximum allowed by the counter design) may occur without adversely affecting the counting rate.

TABLE III

EFFECT ON RESPONSE PROBABILITY OF VERTICAL SOURCE DISPLACEMENT

Position	Observed counting rate (counts/min.)
Source central facing upwards	39,070 ± 140
Source displaced 1 mm. towards top anode facing upwards	39,170 ± 140
Source central facing downwards	39,240 ± 140
Source displaced 1 mm. towards bottom anode facing downwards	39,280 ± 140

(iv) *Effect of Variation of Gold Thickness of Source-mount Films*

The effect on counter response probability of variation of gold thickness on films has been investigated in two sets of experiments, one each for Ni^{63} and P^{32} radiation. Sets of VYNS films (all of 5–10 μgm./cm.^{2} superficial density and mounted over diaphragm apertures of 5 cm. diameter) were coated on one side with a range of gold thicknesses. The superficial density of gold applied was measured spectrophotometrically as described previously (24). For each film, a source was applied centrally to the side of the film on which the gold had been deposited. The high voltage characteristic at a constant discriminator bias setting obtained with this arrangement was recorded. A diaphragm with aperture diameter of 2.5 cm. was then placed over the first, forming effectively a system in which a film with the same gold superficial density was mounted over a smaller aperture. The characteristic curve was again determined. The film, on the original diaphragm, was then removed from the counter and 10 μgm./cm.^{2} gold distilled on to it. In one half of the experiments the gold was

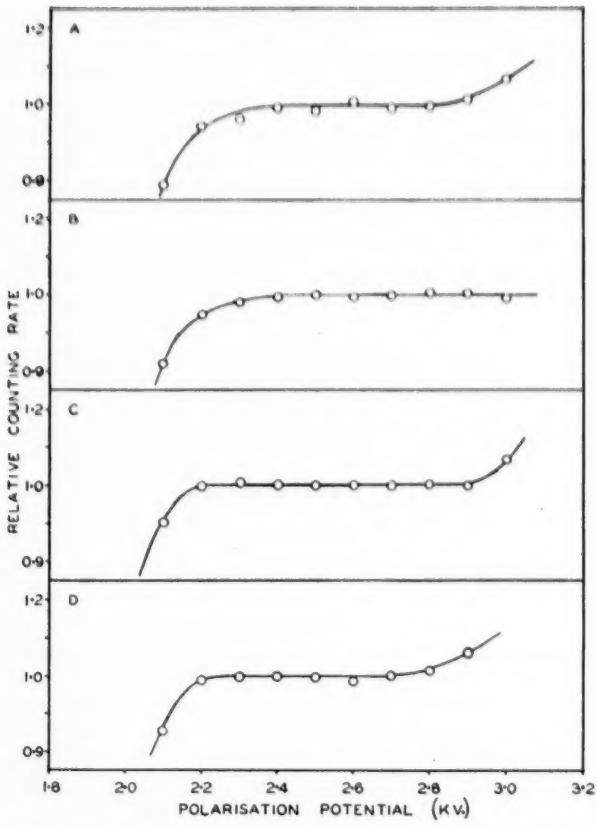


FIG. 11. High voltage characteristics as a function of side of mount to which gold is applied.
A. 5 μgm./cm.² of gold on source side. Ni^{63}
B. 5 μgm./cm.² of gold on reverse side. Ni^{63}
C. 5 μgm./cm.² of gold on source side. P^{32}
D. 5 μgm./cm.² of gold on reverse side. P^{32}

applied to the side of the film carrying the source and in the other half to the reverse side. The voltage characteristic was again determined. Finally, a further 10 μgm./cm.² of gold was applied, this time to the opposite side to the above (both sides now had at least 10 μgm./cm.² of gold) and a fourth characteristic was measured.

The results of the effect of gold thickness are as follows:

1. In all cases the last two characteristics were identical. Identical voltage characteristics were obtained regardless of whether the gold was on the source side, reverse side, or both sides. Representative characteristic curves are shown in Fig. 11.

2. The appearance of the characteristics obtained with increasing thicknesses of gold is shown in Fig. 12 for Ni^{63} and Fig. 13 for P^{32}. In order to render

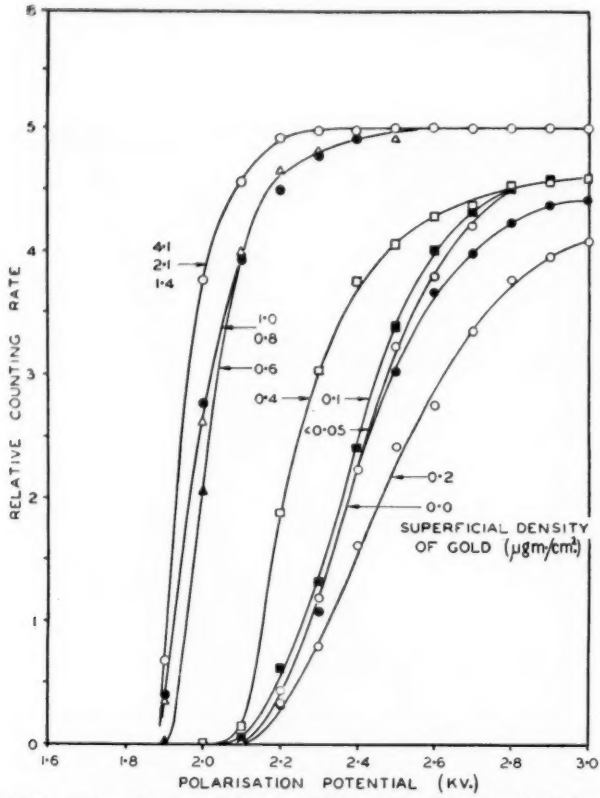


FIG. 12. High voltage characteristics for Ni^{63} as a function of gold thickness on source mount.

the data comparable the counting rates are expressed as a fraction of that found for the source after application of 10 μgm./cm.² of gold to the source mount. The figures plotted apply to a diaphragm aperture of 5 cm. The smaller aperture of 2.5 cm. had the effect of displacing the characteristic for a given gold thickness to a higher polarization potential. However, the gold thickness at which normal characteristics occurred (> 2 μgm./cm.²) was the same for both aperture sizes.

(v) *Effect of Purity of Counting Gas*

The effect of using a counter gas of inferior quality is shown in Fig. 14. This shows a series of characteristics using Ni^{63} as the radioactive source and Technical grade methane as the counting gas. The mass spectrometric analysis of a sample of C.P. methane, giving normal performance, is shown in Table IV. We do not give an analysis for Technical grade methane as this varies from one cylinder to another; the variation in quality is such that some samples may be found to give satisfactory characteristics.

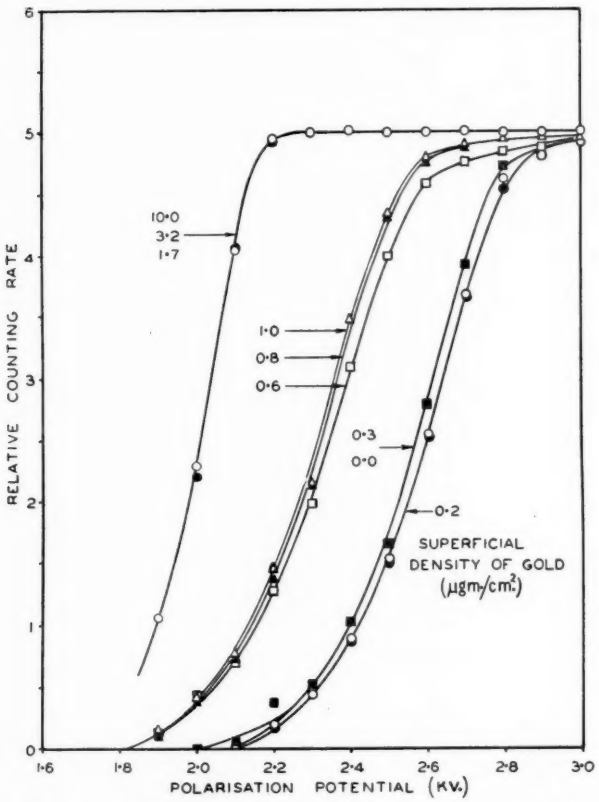


FIG. 13. High voltage characteristics for P^{32} as a function of gold thickness on source mount.

TABLE IV

MASS SPECTROMETRIC ANALYSIS OF C.P. METHANE*

Constituent	Concentration (% by weight)
H_2	0.1
C_2H_4	0.01
C_2H_6	0.59
N_2	0.5
O_2	0.01
CO_2	0.15
C_3, C_4	0.01
CH_4	Remainder

*The authors wish to thank Dr. H. I. Schiff for having performed this analysis.

Experiments performed with synthetically prepared gas mixtures showed that the counter is relatively insensitive to the presence of higher saturated

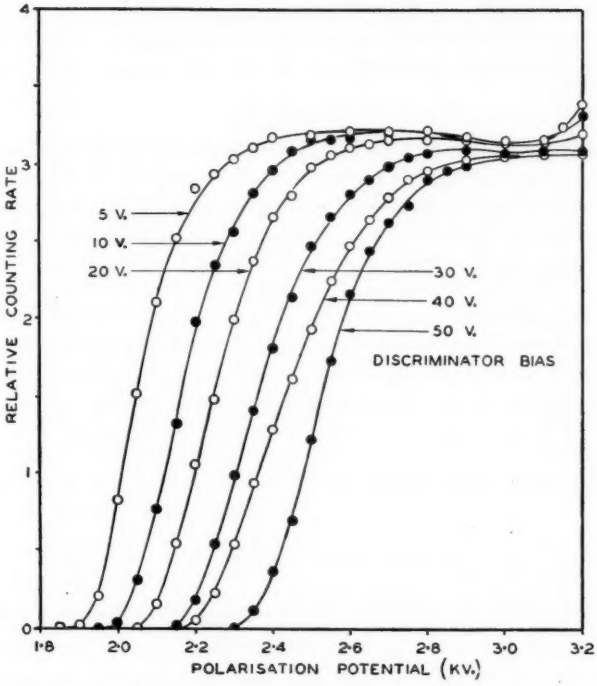


FIG. 14. High voltage characteristics obtained with impure methane for Ni^{63} β radiation.

and unsaturated hydrocarbons in the gas mixtures. The presence of small quantities of air or oxygen, however, is injurious, possibly owing to the electron affinity of the latter.

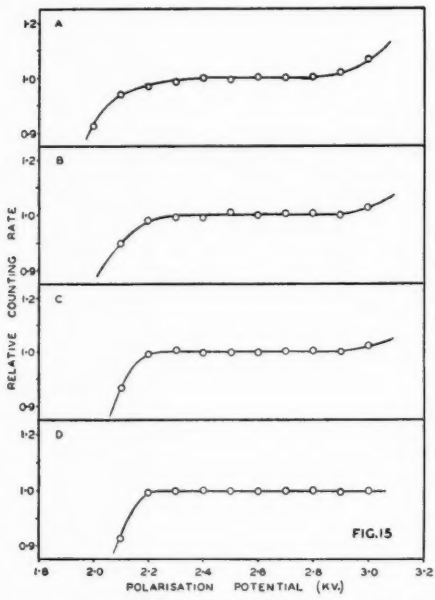
(*b*) "*Counter Geometry*"

Experimental techniques for determining effect of "counter geometry" are described in section (iv) of (*a*).

Characteristics for Ni^{63} and P^{32} are shown in Fig. 15 for both large and small diaphragm apertures. The gold thicknesses used in this experiment were greater than the limit at which regular behavior commenced.

Several workers (16, 23) have discussed the effect of using a relatively thick metal diaphragm in order to support the source-mounting film or foil. Calculations have been made of the fraction of particles emitted which will be "lost" owing to collision with the diaphragm aperture wall. Experiments have also been performed at a single value of the polarization potential to determine the effect of reduction of aperture size.

Fig. 15 shows clearly that no decrease in counter response occurs when the diaphragm aperture diameter normally used is decreased by one half. Provided that a particle produces the necessary minimum number of ions before being absorbed in the aperture wall, no loss in counts will occur. As the



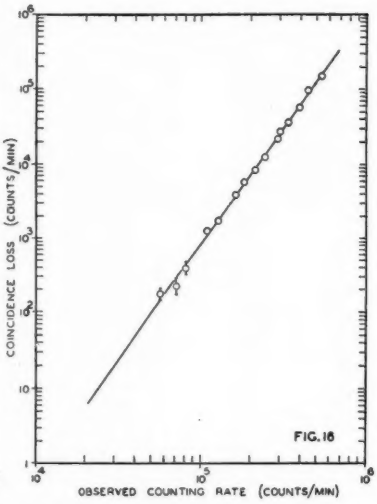


FIG. 15. High voltage characteristics obtained with varying size of diaphragm aperture.
A. 2.5 cm. diameter aperture. Ni^{63}
B. 5.0 cm. diameter aperture. Ni^{63}
C. 2.5 cm. diameter aperture. P^{32}
D. 5.0 cm. diameter aperture. P^{32}

FIG. 16. Coincidence loss function of the counter, at 2.6 kv. high voltage and 15 v. discriminator bias.

polarization potential is increased, the probability of this minimum number being produced approaches unity. Thus, the effect of a reduced aperture size is to displace the polarization characteristic to higher polarization potentials and does not reduce the effective counter geometry from 4π steradians.

(*c*) *Response Probability at Higher Counting Rates*

The coincidence loss function of the counting arrangement has been determined using the method previously described to examine the effect of disintegration rate on the characteristic curve of the counter. A series of 12 sources of P^{32} with disintegration rates ranging from between 3×10^2 and 10^4 d.p.s. was prepared and the counting rates of the single and laminated sources obtained. The results, expressed as "coincidence loss" as a function of the observed counting rate, are shown in Fig. 16 plotted on a log–log scale. Some points for the curve were also obtained by studying the departure from exponential decay of a source of Na^{24}.

The results obtained in Fig. 16 clearly illustrate the loss in counting rate of this counter assembly with increasing disintegration rate. The presentation of the data on a log–log plot results in a curve which is linear and thus facilitates extrapolation to lower counting rates. This correction curve can be used with

confidence for our arrangement up to an apparent counting rate of 6×10^5 c.p.m. It is highly probable that most of the losses which occur are not primarily due to the counter but due to the electronic equipment.

SUMMARY

On the basis of the experimental work discussed in this paper, it is estimated that the error due to the departure of the response probability of a 4π-counter from a value of unity may be reduced to less than 0.1%. For our apparatus, the conditions under which this is realized are the following: source-mounting film of 5–10 μgm./cm.^{2} VYNS resin mounted over a diaphragm aperture of 2.5 or 5.0 cm. and coated with at least 2 μgm./cm.^{2} of gold, counting gas C.P. methane, polarization potential 2.6 kv., and discriminator bias 15 v.

The absence of perceptible slope on the high voltage characteristics of the chamber when functioning correctly allows a considerable latitude in the conditions under which the system may be satisfactorily operated. It further allows a rapid verification that the system is so operating. If an increase of 200 v. in the polarization potential or 10 v. decrease in the discriminator bias causes no change in counting rate observed, then a response probability close to a value of unity is indicated.

ACKNOWLEDGMENTS

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THE COMPRESSIBILITY OF GASES AT HIGH TEMPERATURES

X. XENON IN THE TEMPERATURE RANGE 0° TO 700°C. AND THE PRESSURE RANGE 8 TO 50 ATMOSPHERES[1]

By E. Whalley, Y. Lupien, and W. G. Schneider

ABSTRACT

The virial coefficients of xenon have been measured in the temperature and pressure range described. The results are compared with previous measurements.

The compressibility and the virial coefficients of a number of gases have been previously measured over a wide temperature range using an expansion technique (5). In the present paper measurements of the virial coefficients of xenon from 0° to 700°C. are reported. Temperatures up to 700°C. were employed in the present measurements in order that the virial coefficients could be extended well above the Boyle point. The pressure ranges covered by the measurements were approximately 4 to 20 atm. at 0°C., 8 to 30 atm. at 50°C., and 8 to 50 atm. at the higher temperatures. The xenon gas used in the measurements was supplied by the Linde Company and contained no krypton or other impurities above the limit of detection by a mass spectrometer (about 0.01%). Frequent checks on its purity were made during the runs. The procedure and method of measurement were the same as described previously (5).

RESULTS

The experimental measurements were fitted to the equation of state

$$PV = A+BP+CP^2 \qquad [1]$$

by the method of least squares described previously (6). The values of the virial coefficients A, B, and C, and their standard errors, are summarized in Table I. The units are amagat units. The standard error of the derived coefficients has been defined previously (6) and it is perhaps worth pointing out that it does not necessarily represent the absolute error. The total number of gas expansions carried out in the measurements at each temperature are also given in the table.

At 0°C. the vapor pressure of xenon is approximately 41 atm. (3). It was not possible to fit the measured data above 20 atm. to a virial equation including the fourth virial coefficient, so only measurements below 20 atm. were retained in the final analysis. At 50°C. only measurements below 30 atm. were retained for the same reason. At higher temperatures the maximum pressure was 50 atm. and at all temperatures except for the 0° isotherm the lowest pressure was 8 atm. or lower. For the 0° isotherm, which presented the greatest difficulty in fitting a series equation, the measured pressures were extended down to 4 to 5 atm. This was accomplished with an auxiliary piston

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TABLE I

VIRIAL COEFFICIENTS OF XENON

	N.R.C. values					M.I.T. values		Amsterdam values	
T, °C.	A	Standard error of A ($\times 10^5$)	10^3B, amagat	10^6C, amagat atm.$^{-1}$	No. of expansions	10^3B, amagat	10^6C, amagat atm.$^{-1}$	10^3B, amagat	10^6C, amagat atm.$^{-1}$
0	1.00667	7	-6.614 ± 0.063	-60.5 ± 2.5	40			−6.952	-34.4_4
25						−5.846	-19.9_6		
50	1.19094	8	-4.840 ± 0.020	-19.00 ± 0.41	18	−4.967	-11.7_3	−4.985	-12.3_2
75						−4.243	-6.7_4	−4.273	-7.1_8
100	1.37520	10	-3.748 ± 0.026	-3.44 ± 0.25	17	−3.646	-3.6_3	−3.679	-4.0_4
125						−3.148	-1.6_1	−3.173	−2.10
150	1.55947	11	-2.811 ± 0.025	$+0.95 \pm 0.20$	17	−2.728	−0.28	−2.742	−0.979
200	1.74373	12	-2.127 ± 0.034	$+1.69 \pm 0.30$	16	−2.038	+1.12		
300	2.11226	15	-1.126 ± 0.010	$+1.93 \pm 0.10$	24	−1.058	+1.78		
400	2.48079	17	-0.484 ± 0.020	$+1.80 \pm 0.17$	26				
500	2.84932	20	-0.006 ± 0.014	$+0.80 \pm 0.12$	30				
600	3.21785	22	$+0.357 \pm 0.011$	—	24				
700	3.58638	25	$+0.638 \pm 0.022$	—	24				

gauge which was designed to operate in the pressure range from 3 to 15 atm. The additional low pressure data, which are very essential for obtaining accurate second virials for highly imperfect gases, greatly facilitated the fitting of the data to a series equation. Moreover, for the isotherms between 0° and 150°, where the curvature of the pressure-ratio vs. pressure plots was greatest, the value of N, the volume ratio of the gas pipettes, was determined separately at each temperature by measurements with helium in the usual way. This further simplified the fitting of the data since the intercept on the zero pressure axis of the pressure-ratio vs. pressure plots is then fixed, and the uncertainty of extrapolation of the data from higher pressures is greatly reduced.

COMPARISON WITH PREVIOUS MEASUREMENTS

The compressibility of xenon has been measured by Beattie, Barriault, and Brierley (1) at M.I.T. in the temperature range 16.65–300°C. and pressure range 20–400 atm., and by Michels, Wassenaar, and Louwerse (4) at Amsterdam in the temperature range 0–150°C. and pressure range 16–2800 atm.

The M.I.T. measurements were fitted (2) to the equation of state

$$[2] \qquad PV = RT+\frac{B_V}{V}+\frac{C_V}{V^2}+\frac{D_V}{V^4}.$$

The second and third virial coefficients B_V and C_V were converted to the corresponding pressure virial coefficients of equation [1] by the relations

$$[3] \qquad A = RT/V_N, \quad B = B_V/RTV_N,$$
$$C = \frac{C_V-B_V^2/RT}{V_NR^2T^2},$$

where V_N is the normal volume. The Amsterdam measurements below a density of 50 amagat units were fitted to the equation

$$[4] \qquad PV = A_\rho+B_\rho\rho+C_\rho\rho^2+D_\rho\rho^4.$$

The second and third virial coefficients were converted to the corresponding virial coefficients of equation [1] by the relations

$$[5] \qquad B = B_\rho/A_\rho, \quad C = \frac{C_\rho-B_\rho^2/A_\rho}{A_\rho^2}.$$

Both sets of values are compared with the present measurements in Table I. Both quoted values of the virial coefficients to several figures more than justified by their accuracy. They have been rounded off to the values quoted in the table.

The normal volumes of xenon deduced from the three sets of measurements are listed in Table II.

Our results are not in very good agreement with either the M.I.T. or the Amsterdam values, though these agree very well with each other. Apart from the 0° and 50° measurements, our second virial coefficients are uniformly about 0.1×10^{-3} amagat units higher than the others. This would appear to

TABLE II

NORMAL VOLUME OF XENON

Beattie *et al.**	22.2652 l./mole	(corrected for 0.14% Kr content)
Michels *et al.*	22.2585 l./mole	from measured
This work	22.2654 ± l./mole	0°C. isotherms

**Calculated by using the Beattie–Bridgeman equation of state which had been fitted to the isotherm measurements at higher temperatures. No measurements at 0°C. were carried out by these authors. Less weight should be placed on this value than on the others.*

indicate that there is some systematic difference between the measurements. In both the Amsterdam and the M.I.T. measurements the lowest pressures measured were rather high, being from 16.5 to 27.7 atm. for the Amsterdam isotherms, and 20.7 to 46.1 atm. for the M.I.T. isotherms, whereas the lowest pressure used in the present measurements was never greater than 8 atm. Since the second virial coefficient is obtained from a zero pressure slope it would seem that greater accuracy could be obtained from measurements extending to lower pressures. Because xenon is a rather imperfect gas, particularly at the lower temperatures, the measurements should preferably not extend to a too high pressure. The deviations of the experimental from the observed values of $V(PV-RT)$ at constant temperature of Beattie's measurements are not random (Table I, Ref. 2). We have not reported our pressure measurements since they are partly functions of the apparatus, but care was taken in the analysis that the deviations of the calculated from the observed pressure ratios were random, indicating that equation [1] was a good fit to the data. Another contributing cause of the different virial coefficients may be the use of an inverse volume series for the equation of state in the M.I.T. and the Amsterdam measurements, whereas we have used a pressure series.

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IDENTIFICATION OF SPRUCE SULPHITE LIQUOR COMPONENTS[1]

By E. A. Kvasnicka and R. R. McLaughlin

ABSTRACT

Spruce sulphite liquor was extracted with butyl acetate. By means of solvent fractionation, chromatography, and countercurrent distribution, vanilloyl methyl ketone, vanillin, dihydroconiferyl alcohol, 3,3′-dimethoxy-4,4′-dihydroxystilbene, and a new phenolic compound, $C_{20}H_{24}O_5$, were isolated and identified.

In the field of lignin chemistry, the oxidation of sulphite waste liquor has aroused considerable interest. A large amount of work has been carried out for the purpose of isolating and identifying the low molecular weight fragments of lignin in such oxidized liquors (22). However, no such comparable effort has been made to investigate the original sulphite waste liquor, though a few constituents have been noted (8, 16, 21, 28). The amount of such compounds is much smaller than in oxidized liquor and consists mainly of degradation products of lignin together with some materials derived from the wood, or their reaction products (32, 35). It follows that the identification of these components could be an important link between the studies of wood extractives and the examination of the constituents of oxidized liquors, and might indicate technical uses of the compounds so identified, or of compounds that could be derived from them.

These considerations induced a thorough investigation of the solvent-extractable components of spruce sulphite liquor in this laboratory. The present paper describes the first part of the results obtained.

Spruce sulphite liquor was extracted with butyl acetate, which removes a larger amount of solids than benzene or ether (11). The extract contains three main groups of components: tannins and conidendrin, other phenols, and neutral compounds (hydrocarbons and esters). It was necessary to perform a crude separation into these groups by solvent fractionation before the further work was done. Conidendrin was separated by its low solubility in ethanol. Extraction of the ethanol-soluble part with ether left a minor amount of conidendrin and the tannins as residue. Extraction of the ether-soluble part with petroleum ether removed mainly hydrocarbons and left as residue the main part of the other phenolic compounds.

The next step was the separation of these groups into the bisulphite-soluble compounds (extraction with 21% sodium bisulphite), acids (10% sodium bicarbonate), and phenols (6% sodium hydroxide). The neutral compounds and a very small amount of phenols with extreme pK values (2) remained after these extractions.

The large number of components in these fractions made separations difficult and required an efficient method to check the course and extent of

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Contribution from the Department of Chemical Engineering, University of Toronto, Toronto 5, Ontario.

the subsequent steps. Paper chromatography was used extensively for this purpose.

The bisulphite-soluble material was separated by chromatography. Vanillin and 1-(3-methoxy-4 hydroxyphenyl)-propanedione-1,2 were isolated. The latter compound has not yet been reported in spruce sulphite liquor. Its presence in the aqueous filtrates of lignin ethanolysis mixtures (3, 14) shows some relation between the degradation processes during ethanolysis and sulphite cooking.

The alkali-soluble material was separated by fractional acidification, utilizing the difference of the pK values. Further separation was obtained by countercurrent distribution which yielded dihydro-coniferyl alcohol (3-(3-methoxy-4-hydroxyphenyl)-propanol-1) as a new component of spruce sulphite liquor. Hibbert (13) and Schuerch (30) found this compound in the hydrogenolysis products of maple lignin. Kawai (17) isolated it as a degradation product of egonol.

From the same fraction, a new phenolic compound, $C_{20}H_{24}O_5$, m.p. 134°C., was isolated. Its spectrum (UV, IR) is closely related to dihydroconiferyl alcohol and the investigation of its structure is under way.

In addition to the previously reported compounds, a small amount of 3,3′-dimethoxy-4,4′-dihydroxystilbene was isolated by chromatography of the alkali-soluble fraction. Richtzenhain (26) found this stilbene in oxidized sulphite liquors and reported its absence in untreated liquors.

It can be seen that the compounds now isolated from spruce sulphite liquor are known as reaction products of quite different lignin degradation procedures: ethanolysis, hydrogenolysis, and oxidation.

Further investigations will be concerned with the identification of the remaining compounds of the extracts and with the origin of the isolated products.

EXPERIMENTAL PART

1. *Extraction*

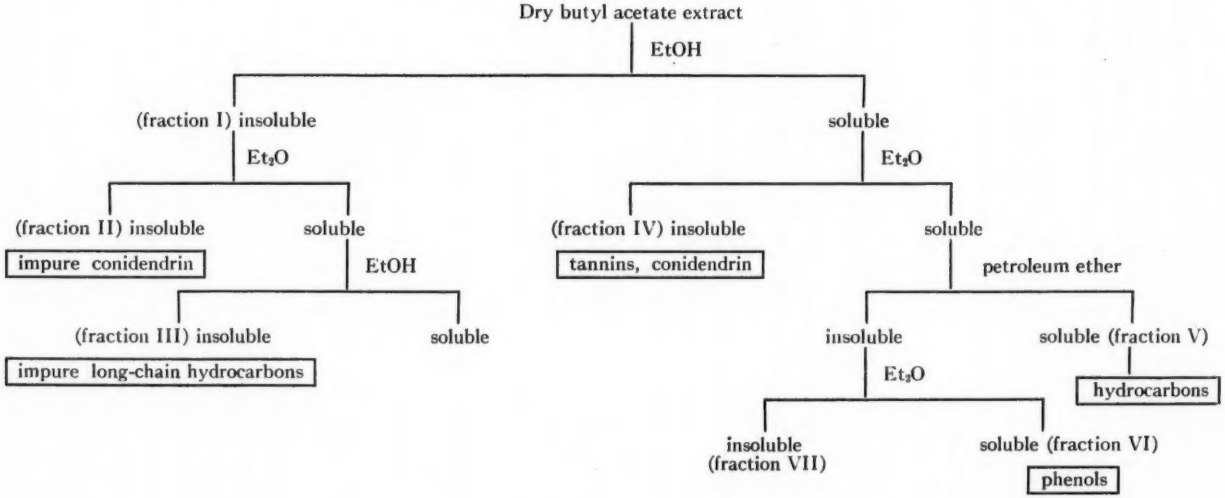
Spruce sulphite waste liquor (SWL) (Cook No. 3001/48, Ontario Paper Co., Thorold, Ont.) was extracted with *n*-butyl acetate by a 21-ft. fast spray type extraction column (4) (countercurrent flow, flow ratio 2: 1/BuOAc–SWL (continuous phase)). This extract was concentrated and the last part of the solvent was removed in a 3-liter glass still under reduced pressure (12 mm., 40°C. bath temp.). Several such extractions gave an average of 1.38 gm. dry extract per liter of sulphite liquor. This amount can be increased at lower flow rates.

2. *Solvent Fractionation* (See Table I)

Fifty grams of dry extract was dissolved in 500 ml. ice-cold dry ethanol. The insoluble material (fraction I) was filtered off. The ethanol was removed under reduced pressure (12 mm.). The residue was shaken twice with 100 ml. portions of ether (30 min.). The insoluble part (fraction IV) was separated. The ether was distilled off under reduced pressure. The residue was extracted

TABLE I

SOLVENT FRACTIONATION OF BUTYL ACETATE EXTRACT



NOTE: Fraction	II	III	IV	V	VI	VII	others and loss
Weight (as % of the dry butyl acetate extract)	12.6	1.3	27.9	17	37.9	0.7	2.6

twice with 100 ml. portions of petroleum ether (b.p. 30–60°C.). The insoluble part (fraction V) was separated and extracted twice with 75 ml. portions of ether. The residue (fraction VII) was filtered off. Fraction I, the ethanol-insoluble material, was shaken with 200 ml. ice-cold ether (five minutes) and filtered. The residue was impure conidendrin (fraction II). The ether filtrate was concentrated to 50 ml. under reduced pressure, cooled to 0°C., and 150 ml. ice-cold dry ethanol added. The resulting precipitate was impure melene (28) (fraction III).

The extractions and separations reported under parts 1 and 2 are based on parts of the theses submitted by Sacks (28) and Kenyon and Kerr (18) in partial fulfillment of the requirements for the M.A.Sc. degree and B.A.Sc. degree at this University.

3. *Group Separation*

After evaporation fractions V and VI were each dissolved in 20 times their volume of butyl acetate. A small amount of conidendrin (1.6 gm.) separated from fraction VI after this procedure.

The solution was extracted successively with sodium bisulphite solution (21%), sodium bicarbonate solution (10%), and sodium hydroxide solution (6%).

The sodium bisulphite extract was acidified with sulphuric acid, heated, aspirated, and cooled. It was extracted with chloroform.

The bicarbonate and sodium hydroxide solutions were also acidified and extracted with chloroform.

The remaining butyl acetate solution was distilled under reduced pressure until the solvent was removed.

The chloroform solutions of the other groups were also evaporated and the weights of extracted materials determined, as shown in Table II.

TABLE II

GROUP SEPARATION OF FRACTIONS V AND VI

Group:	Bisulphite-soluble	Bicarbonate-soluble	Sodium-hydroxide-soluble	Remaining fraction
Fraction V	0.09	0.21	0.42	16.3%
Fraction VI	3.0	0.9	24.0	10 %

NOTE: *All numbers represent per cent by weight of the original dry butyl acetate extract.*

4. *Bisulphite-soluble Compounds*

Paper chromatography (see part 6) was used to give a preliminary indication of the composition of the fraction. No significant differences were found between the bisulphite-soluble components of fractions V and VI. Both contain two principal compounds (compound A—R_F: 0.53, compound B—R_F: 0.73) and minor amounts of other compounds.

Compound A was isolated from a series of paper chromatograms on Whatman No. 3 paper with the same solvent system (see part 6). The position of the spots was detected under the UV lamp, the spot area was marked, and was

cut out. Elution of the spots obtained by chromatography of a total amount of 12 mgm. bisulphite fraction gave 2.5 mgm. of a yellow oil. UV and IR spectra indicated a diketo-compound of the guaiacyl group. A larger quantity of bisulphite-soluble material (200 mgm.) was used for a chromatogram on a 2 × 15 cm. column of 100 mesh silica gel (Mallinckrodt). After development with 600 ml. benzene–methanol (250: 1, v/v), the column material was cut into zones. The material in the yellow zones was eluted with methanol and checked by paper chromatography. The combination of the material of the corresponding zones gave after recrystallization from ethanol–water (1: 3), 21 mgm. of yellow crystals, m.p. 72.5°C.[2]

The mixed melting point with authentic vanilloyl methyl ketone was not depressed. The spectra of the compounds were identical and both gave the same R_F values, color reactions with spray reagents, and the same characteristic nickel salt of the dioxime.

IR spectrum:[3] Maxima at 1720, 1664, 1593, 1510, 1463, 1430, 1376, 1350, 1297, 1262, 1227, 1202, 1172, 1139, 1125, 1117, 1033 cm.$^{-1}$ (CS_2, CCl_4)

The UV spectrum of compound B and its R_F value were identical with those of vanillin. Sublimation of a 50 mgm. sample of bisulphite-soluble material under reduced pressure gave 20 mgm. of crystalline sublimate, identified as vanillin by mixed melting point and paper chromatography.

5. *Sodium-hydroxide-soluble Compounds*

Paper chromatography (see part 6) revealed only minor quantitative differences in the composition of the sodium hydroxide extracts of fractions V and VI. Aside from conidendrin, two principal components (compound C—R_F: 0.20, compound D—R_F: 0.55) and smaller amounts of about 20 other phenolic compounds were indicated.

The course of all the following separation steps was checked by paper chromatography.

Half of the alkali-soluble part of fraction VI was dissolved in 200 ml. 2 *N* sodium hydroxide and the pH adjusted to 12.0 by addition of sodium dihydrogen phosphate. After extraction with chloroform (50 ml.), the water phase was acidified further in steps of 0.25 pH units and extracted again after each step with 50 ml. chloroform, until pH 5 was reached.

Isolation of Compound D

The five chloroform extracts made between pH 12.0 and 11.0 contained almost all of compound D and also some other phenols. These extracts were concentrated to a volume of 15 ml. The further separation was effected by countercurrent distribution between chloroform–ethanol – phosphate buffer solution (pH 12) (29) in a 24 stage circular type Craig apparatus (5). (Volume relation 7.7: 12 (buffer phase)). After 24 transfers, compound D had migrated from tube 1 to tubes 6–9 and these fractions contained only minor amounts of impurities. The solvent of these fractions was removed under reduced pressure

[2]*All melting points are uncorrected.*

[3]*We are grateful to Miss E. M. Kirby of the Ontario Research Foundation for the determination of all infrared spectra.*

(bath temperature below 50°C.) and the residue dissolved in the minimum amount of hot benzene. After cooling, crystals separated (240 mgm.). Recrystallization from benzene gave colorless prismatic crystals, m.p. 134°C.

Analysis: Found: C: 69.98, 69.95; H: 7.05, 6.96; CH_3O: 17.83, 17.90
Calc. for $C_{20}H_{24}O_5$: C: 69.74; H: 7.03; CH_3O: 18.1

Mol. wt.: Found: (Rast): 340
Calc.: 344.39

UV spectrum: (95% EtOH): Max. 229 mμ, 283 mμ
(95% EtOH, 1 mole KOH/l.): max. 247 mμ, 299 mμ

IR spectrum: Maxima at: 1604, 1516, 1453, 1430, 1366, 1266, 1231, 1202, 1180, 1149, 1120, 1094, 1030 cm.$^{-1}$ (melt, supercooled)

The mother liquors of these crystallizations yielded further quantities of compound D after chromatography on Magnesol–Celite (5: 1) (23) with benzene–methanol (250: 1) as eluent.

Isolation of Compound C

The chloroform extracts made between pH 10.75 and 9.75, containing the principal amount of compound C, were concentrated to 15 ml. and subjected to countercurrent distribution in a manner similar to that described above. Solvent system: chloroform – phosphate buffer (pH 11) – ethanol–water (29): volume relation 7.7: 14 (buffer phase). After 34 transfers, the compound had migrated from tubes 1–2 to tubes 18–28. The purest fractions were combined, the solvent removed under reduced pressure, and the residue purified further by chromatography on a Magnesol–Celite (5: 1) column (23) using benzene–methanol (250: 1, v/v) as eluent. The fractions were combined according to the results of paper chromatography. The solvent was removed under reduced pressure and a colorless oil remained (400 mgm.). Its IR spectrum was very similar to the spectrum of eugenol.

The compound was now benzoylated. A sample, 365 mgm., was dissolved in 10 ml. pyridine, cooled, and 0.5 ml. benzoyl chloride added. After 24 hr. at room temperature, the mixture was heated on the steam bath for five minutes, diluted with water, and extracted with chloroform. After drying, the solvent was removed and 720 mgm. crude benzoyl compound isolated. Recrystallization from petroleum ether – benzene yielded white needles, m.p. 62–63°C. A synthesized sample of dihydroconiferyl alcohol dibenzoate gave no depression of the mixed melting point.

Comparison of compound C with a synthesized sample of dihydroconiferyl alcohol (30) gave identical R_F values, spectra, refraction indices, and color reactions.

UV spectrum: (95% EtOH): Max. 228 mμ, 281.6 mμ

IR spectrum: Maxima at 1608, 1513, 1464, 1431, 1368, 1266, 1231, 1202, 1183, 1149, 1119, 1050, 1037 cm.$^{-1}$ (CS_2, CCl_4)

Isolation of 3,3′-Dimethoxy-4,4′-dihydroxystilbene

The alkali-soluble part (5 gm.) of fraction VI was chromatographed on a Magnesol–Celite (5: 1) column (23) (size: 3 × 40 cm.) using benzene–methanol

(200: 1) as eluent. The effluent was collected in 15-ml. portions. In addition to the compounds reported above a strongly fluorescent compound was found in the first fractions (Nos. 6–10) of the effluent. Another chromatogram was made on silica gel (Mallinckrodt, 100 mesh), using the same eluent. The zone with a strong violet fluorescence was cut out, eluted with ethanol, and concentrated. The resulting crystals were collected and recrystallized from ethanol, giving 20 mgm. colorless needles, m.p. 213°C. The mixed melting point with 3,3′-dimethoxy-4,4′-dihydroxystilbene (26) was not depressed. The color reactions were also identical.

6. *Paper Chromatography*

The solvent systems: butanol – 2% ammonia (24), butanol–water and benzene – petroleum ether – water (19) were used to some extent. However, the major part of paper chromatography in this work was carried out with the solvent system: tetrahydrofuran – petroleum ether (b.p. 65–110°C.) – water (3: 7: 5) because of the short travel time for this solvent (about 10 cm. per hour) and the good separation of a large number of compounds (see Table III). Descending chromatography was used.

TABLE III

IDENTIFICATION OF PHENOLIC COMPOUNDS BY PAPER CHROMATOGRAPHY

Compound (Reference)	$100 \times R_F$	Color reaction:		UV fluorescence on pH 11 paper
		Diazotized sulphanilic acid	Diazotized *p*-nitraniline	
Acids				
Vanillic	13	Orange	Purple	
Homovanillic (7)	9	Red	Gray-blue	
Hydroferulic (6)	20	Red	Gray-blue	
Ferulic (27)	11	Purple	Lilac	Blue
Syringic	4	Pink	Blue	
Keto compounds				
Vanillin	53	Pale orange	Purple	Dark violet
Acetovanillone	48	Pale orange	Purple	Bright violet
Propiovanillone (10)	67	Pale orange	Purple	Bright violet
R—CO—CO—CH_3 (14)	73	Orange-brown	Red-brown	Dark brown
α-Hydroxy-propiovanillone (15)	32	Orange		Blue
R—CH_2COCH_3 (33)	52	Orange-red		
Alcohols				
Vanillyl alcohol	6	Orange		
Apocynol (9)	17	Orange		
R—$CH(OH)C_2H_5$ (31)	39	Orange		
R—$CH_2.CH(OH).CH_3$ (31)	26	Red	Gray-lilac	
R—$(CH_2)_3OH$ (30)	20	Red	Gray-lilac	
Coniferyl alcohol (1)	7	Purple	Lilac	

(*R* = *3-MeO-4-HO—*C_6H_2-)

Paper: Whatman No. 1

Spray reagents: 2,4-dinitrophenylhydrazine (0.4% in 2 *N* hydrochloric acid)
ferric chloride (1%) – potassium ferricyanide (1%), 1:1
phosphomolybdic acid (4%), followed by treatment with ammonia vapors
diazotized sulphanilic acid (12)
diazotized *p*-nitraniline (34)

Buffered papers: sheets of Whatman No. 1 paper were sprayed with 2% phosphate buffer solutions of pH values between 4 and 10 and dried at room temperature. These papers made possible separations of acids and acidic phenols from each other and from other phenolic compounds with similar R_F values. The R_F values of acidic compounds decrease on the papers with increasing alkalinity gradually to zero, depending on their pK values (20). This method can be used to distinguish between vanillin, acetovanillone, and vanillyl methyl ketone or between hydroferulic acid and dihydroconiferyl alcohol. The method of Reid and Newcombe (25) was also used to distinguish between vanillin and acetovanillone.

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A NEW TYPE OF CELLULOSE ETHER

THE PREPARATION AND PROPERTIES OF THE ω-(*p*-AMINOACETOPHENONE) ETHER OF CELLULOSE[1]

By R. R. McLaughlin and D. B. Mutton[2]

ABSTRACT

With the object of preparing a cellulose ether containing diazotizable amino groups, seven different forms of cellulose, or cellulose derivatives, were treated with 10 different reagents under a variety of conditions. Reasons why only one reagent under one set of conditions gave a product with a substantial degree of substitution (0.35) and with satisfactory physical properties are presented. This resulting ether, the ω-(*p*-aminoacetophenone) ether of cellulose, was prepared by the reaction between ω-chloro-*p*-aminoacetophenone and ethanol-washed soda-cellulose (from wood pulp, cotton, paper, or rayon). It can be (*a*) diazotized and coupled with any of the usual reagents to yield cellulose-azo colors, (*b*) xanthated, before or after coupling, and spun as rayon filament; if uncoupled at this point it can be diazotized and coupled, (*c*) dyed directly with acid dyes, (*d*) rendered organo-soluble by nitration, and (*e*) carboxymethylated after coupling to yield colored, water-soluble derivatives. It is insoluble in the usual cellulose reagents, and is as stable to acid and alkaline hydrolysis as cellulose.

INTRODUCTION

The purpose of the work described in this paper was the preparation of cellulose ethers containing primary aromatic amino groups. On diazotization and coupling such ethers should produce azo compounds in which the chromophore groups form part of the molecule of the cellulose derivatives, which should be as stable as cellulose to most reagents. Analogous nitro compounds, because they may be reduced to the corresponding aromatic amines, are also of interest. Ethers rather than esters were investigated because the former are more resistant to hydrolysis.

A number of attempts to prepare colored cellulose derivatives have been reported. In 1926 Peacock (42) obtained what he regarded as a superficial coloring of cotton by treating it with *m*-nitrobenzyl-phenyldimethyl-ammonium chloride, diazotizing, and coupling. A similar result was obtained by Kursanov and Solodkov (33). Charles Gränacher (19, 20, 21, 22, 23) obtained diazotizable cellulose fibers by a superficial etherification of swollen cellulose (alkali cellulose washed free of alkali with water) with such reagents as *p*-nitrobenzyl chloride and 2,4-dinitrochlorobenzene in the presence of lithium carbonate. Repetition of this work by us failed to give a degree of substitution (D.S.) greater than 0.01. A modification of this method applied by Pancirolli (41) to cellulose and starch yielded a *p*-nitrobenzyl ether of starch with a D.S. of 0.04. Repetition of Pancirolli's work led to the successful method described later in this paper.

The usual etherification reaction between alkali cellulose and an organic halide forms the basis for a number of patents (7, 8, 9, 10, 11, 28) wherein

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Contribution from the Department of Chemical Engineering, University of Toronto, Toronto, Ontario. Abstracted from the Ph.D. thesis of D. B. Mutton.
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reaction conditions are usually described only vaguely, and analytical data are usually lacking. Such an attempt by Niethammer and Konig (39) with 2,4-dinitrochlorobenzene yielded a product with nitrogen content below 1% (D.S. <0.06). Reduction, diazotization, and coupling gave colors that were not intense. Repetition of this work by Andrews, Hyer, and Ray (1), in this laboratory, failed to give any product with a D.S. above 0.04, and variation of the reaction conditions with this reagent by us gave essentially the same results.

Recently Guthrie (25) colored cotton by treating it with an alkaline solution of a dye containing the chloroethyl or sulphatoethyl group, but concluded from the colors obtained that the D.S. was very low (26).

Seiberlich (49), Dinklage (9), and others (28) have reported the formation of a diazotizable cellulose derivative by the nitration of benzyl cellulose and reduction of the product, but the nitrogen content was not given. Our repetition of this work gave inconclusive analytical results and no intensely colored products.

Several other patents (12, 13, 50) refer to diazotizable cellulose ethers but no analytical results are cited.

Though the present work was confined to ethers, it ought to be recorded here that colored cellulose esters have been reported by a number of workers (3, 9, 10, 13, 24, 29, 30, 45), also a diazotizable aminocellulose (47, 48), and several derivatives prepared from oxycellulose (38, 46).

This survey indicates that no well-defined cellulose ether containing aromatic amino or nitro groups with a D.S. > 0.05 has hitherto been prepared. Apparent degrees of substitution below 0.05 might well be ascribed to adsorption.

RESULTS AND DISCUSSION

Preliminary Experiments

Several different forms of cellulose were treated with a number of etherifying agents under a variety of conditions. The etherifying agents used were 2,4-dinitrochlorobenzene, *p*-nitrochlorobenzene, picryl chloride, *p*-chloroaniline, *p*-bromoaniline, *p*-nitrobenzyl chloride, *p*-nitrobenzyl bromide, β-(*o*- and *p*-nitrophenyl) ethyl bromide, γ-(*p*-nitrophenyl) propyl bromide, *p*-amino-ω-chloroacetophenone, and *p*-nitrobenzyl chloromethyl ketone.

Three principal forms of cellulose were used. (1) Cellulose activated by swelling or solution was treated with an etherifying agent in the presence of some acid-binding reagent or solvent. This group included "activated cellulose", prepared according to Gränacher (19, 20, 21, 22, 23), and organo-soluble cellulose acetate, D.S. (of acetyl groups) 1.75 to 2.67. (2) Benzyl cellulose (D.S. 1.0 in benzyl groups, remaining hydroxyl groups blocked by acetylation) was nitrated according to Seiberlich (49) and Dinklage (9). (3) An etherifying agent was treated with an alkali derivative of cellulose such as ordinary alkali cellulose; sodium cellulosate according to the methods of Gaver (17, 18), and of Sugihara and Wolfrom (51); sodium cellulosate from sodium in liquid ammonia (15, 16, 43); and ethanol-washed alkali cellulose, modified after Pancirolli (41). The most commonly used conditions consisted in refluxing a solution (ether,

benzene, dioxan, ethanol, pyridine, or acetone) of the etherifying agent with the particular form of cellulose in use. Some experiments were carried out in the molten etherifying agent, and some at room temperature, with and without shredding, with and without solvent.

Purification of the products was effected by extraction of the unused reagents and the by-products with suitable solvents.

The only cases in which degrees of substitution consistently above 0.05 were obtained were those in which (*a*) molten *p*-nitrobenzyl chloride was reacted with sodium cellulosate (method of Sugihara and Wolfrom) (51), and (*b*) *p*-nitrobenzyl chloride and bromide, and *p*-amino-ω-chloroacetophenone, were reacted with ethanol-washed alkali cellulose.

The reaction with sodium cellulosate produced erratic results, and the products were amorphous powders of low D.P. Using ethanol-washed alkali cellulose, *p*-nitrobenzyl chloride and bromide produced degrees of substitution averaging 0.07–0.10. *p*-Amino-ω-chloroacetophenone, on the other hand, consistently gave products having D.S. between 0.20 and 0.35. This was by far the most successful combination.

In any etherification reaction the driving force, and the solubility, reactivity, and molecular size of the etherifying agent determine the result. For a successful reaction, a sufficient driving force must be available (40) (usually determined by the alkali concentration); the solubility of the reagent must be high enough to permit substantial diffusion to the reaction centers at the cellulose hydroxyl groups; and the reactivity of the reagent must be such that the extremes of a negligible rate of reaction and complete saponification to useless by-products are avoided.

The reason for the negative results obtained with most of the methods and reagents used in this work is probably that all the above conditions were not simultaneously fulfilled in such cases.

When alkali cellulose is washed with ethanol, much of the excess sodium hydroxide is removed, although the sodium hydroxide and water which are bound or preferentially absorbed by the cellulose are not affected to the same extent. Thus, while the proportion of hydroxyl groups in equilibrium with the sodium salt will be slightly decreased (making a lower D.S. possible), the excess alkali which is responsible for the hydrolysis of the etherifying agent will be sharply reduced. This, together with the higher water solubility of *p*-amino-ω-chloroacetophenone (hereinafter referred to simply as "ω"), is likely the reason for the success of the reaction of "ω" with ethanol-washed alkali cellulose.

ω-(p-Aminoacetophenone) Ether of Cellulose

This new ether of cellulose was prepared with D.S. up to 0.35 by reacting ethanol-washed alkali cellulose with an ethanol solution of "ω" (cf. Experimental). The purified product was usually light-yellow and fibrous.

The ratio of sodium hydroxide to the anhydro-glucose units of cellulose was varied by increasing the number of ethanol washings. The effect that this has upon the degree of substitution of the ω-cellulose is shown in Fig. 1, and indicates that the optimum molar ratio is about 1:1.

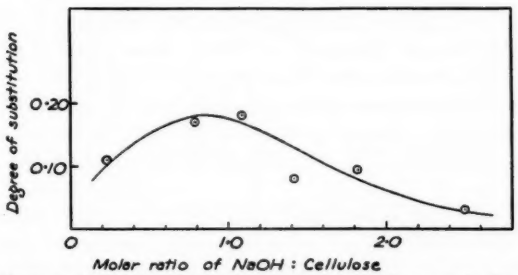

FIG. 1. The effect of the molar ratio of sodium hydroxide to anhydro-glucose units of cellulose upon the degree of substitution of ω-cellulose.

Twenty-eight per cent potassium hydroxide was used for steeping, in place of sodium hydroxide, and the resulting alkali cellulose was washed with ethanol to a potassium hydroxide: anhydro-glucose units molar ratio of 0.66: 1. When treated in the usual way with "ω", the product obtained had a D.S. of 0.20, showing, as was expected, that potassium hydroxide may be used in this reaction.

Since we had concluded that no well-defined cellulose ether containing aromatic amino or nitro groups with a D.S. > 0.05 had previously been prepared, we wished to establish definitely that ω-cellulose was actually a cellulose ether. No one experiment proved this unequivocally. However, the evidence given by the properties of the compound, as described below, make any other interpretation extremely unlikely.

Because of the adsorptive properties of cellulose it was necessary to demonstrate that ω-cellulose is neither a mechanical mixture of cellulose and "ω", nor an adsorption complex. A mechanical mixture of cellulose and "ω" was dialyzed with hot acetone, and the residue was found to be nitrogen-free. On the other hand, the nitrogen content of a sample of ω-cellulose (D.S. 0.28) was unchanged after dialysis for one week with hot acetone.

The stability of ω-cellulose (D.S. 0.21) to alkali with respect to time, temperature, and alkali concentration is shown in Figs. 2, 3, and 4. The levelling-off of all curves at D.S. 0.18 indicates that the ether linkage in ω-cellulose is quite stable to alkali. The slight decrease observed may be due to the removal of some lower molecular weight fractions having a slightly higher-than-average D.S.

In another experiment, boiling with 1% sodium hydroxide solution for six hours lowered the D.S. only from 0.30 to 0.26. The same treatment with 1% sodium carbonate solution had no effect.

The stability of ω-cellulose (D.S. 0.21) to acid with respect to time, temperature, and acid concentration is shown in Figs. 5, 6, and 7. These curves do not level off at a constant D.S. as in the case of the alkaline treatment, but slope gently downward. This indicates that the ether linkage in ω-cellulose is slowly cleaved by acid in a manner analogous to the acid hydrolysis of cellulose. The samples represented by the last two points in Fig. 7 were visibly degraded and could be crumbled to a powder when dry. This experiment indicates either that the ether linkage in ω-cellulose is as stable as the glycosidic linkages

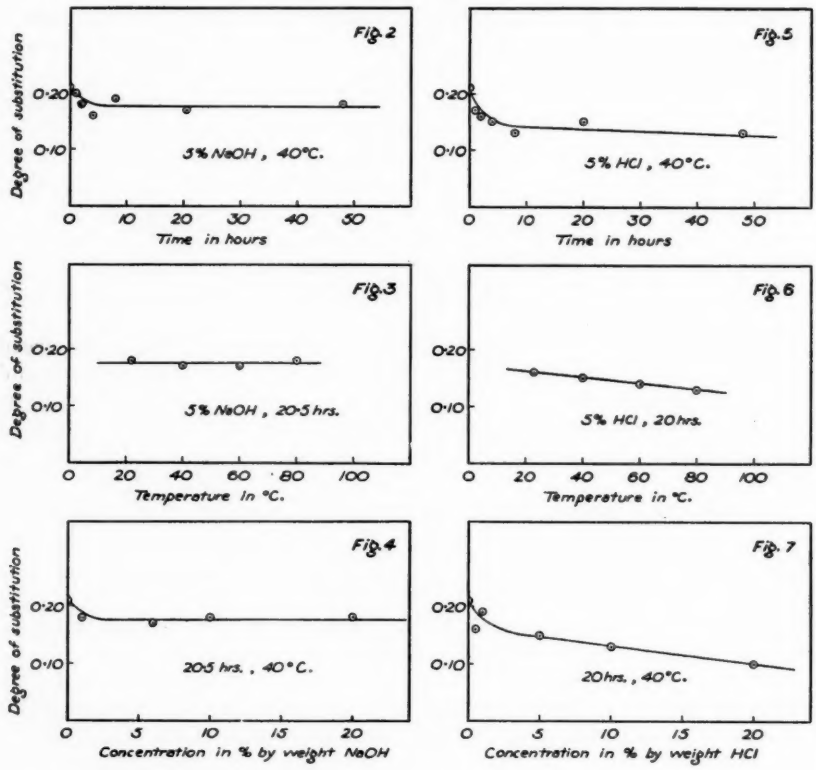

FIG. 2. The effect of time of hydrolysis in 5% sodium hydroxide at 40°C. upon the degree of substitution of ω-cellulose.

FIG. 3. The effect of temperature of hydrolysis in 5% sodium hydroxide for 20.5 hr. upon the degree of substitution of ω-cellulose.

FIG. 4. The effect of concentration of sodium hydroxide during hydrolysis at 40°C. for 20.5 hr. upon the degree of substitution of ω-cellulose.

FIG. 5. The effect of time of hydrolysis in 5% hydrochloric acid at 40°C. upon the degree of substitution of ω-cellulose.

FIG. 6. The effect of temperature of hydrolysis in 5% hydrochloric acid for 20 hr. upon the degree of substitution of ω-cellulose.

FIG. 7. The effect of hydrochloric acid concentration in the hydrolysis at 40°C. for 20 hr. upon the degree of substitution of ω-cellulose.

in cellulose, or else that the low molecular weight portions of the cellulose ether, which probably have a higher D.S., are being hydrolyzed and made water-soluble by the acid treatment.

In another experiment, boiling with 1% hydrochloric acid for six hours did not lower the D.S. (0.30) of a sample of ω-cellulose.

During purification of several batches of ω-cellulose, continued washing with acetone provided a colorless extract. On changing to hot water the washings were again slightly colored (yellow) but presently became colorless.

On changing to acetone the first washings were colored, but also, presently, became colorless. This process could be repeated, apparently indefinitely. During this treatment, the D.S. of the ω-cellulose decreased gradually, in one case from 0.28 to 0.19 over a period of six days' washing. It seems likely that some of the lower-molecular-weight and more highly substituted fraction of the ω-cellulose was slightly soluble in the solvent combination existing when a change of solvent was made.

Samples of ω-cellulose (D.S. 0.33) and the corresponding coupled products were examined by Industrial Cellulose Research, Limited, Hawkesbury, Ont., for the purpose of making viscosity measurements, but were found to be insoluble in cuprammonium or cupriethylene–diamine solutions. Were the product an adsorption complex this behavior would be very unlikely.

It could be envisaged that an initial small D.S. of "ω" groups in cellulose could be followed by a polymerization reaction between the excess "ω" and the amino groups present in the cellulose ether, thus:

Cellulose—O—CH_2.CO.C_6H_4.NH.CH_2.CO.C_6H_4.NH. . . . etc. An analysis for nitrogen would thus indicate a high D.S. whereas it might, in fact, be small. To clarify this point a sample of ω-cellulose (1.53% nitrogen, D.S. 0.21) was treated with nitrous acid at room temperature, heated to boiling, washed, and dried. The product contained 0.52% nitrogen (D.S. 0.07). It thus appears that at least two thirds of the original nitrogen was present as primary amino groups, i.e. that the original D.S. was at least 0.14. This is a minimum value, as it would be higher if there were any introduction of nitroso groups during the treatment with nitrous acid. This point is being further investigated.

Several attempts were made to hydrolyze ω-cellulose and to isolate ω-glucose. Neither the acetolysis procedure of Dickey and Wolfrom (6) nor the methanolysis procedure of Sugihara and Wolfrom (51) produced significant hydrolysis of the ω-cellulose. These failures, however, indicate a significant difference in structure between cellulose and ω-cellulose. Hydrolysis with 72% sulphuric acid was more successful. The hydrolyzate was purified and chromatographed. No ω-glucose was isolated, but a low-molecular-weight carbohydrate fragment containing an aromatic amino group was detected. Investigation of this problem is continuing.

Dr. A. W. Pross of the Central Research Laboratory of Canadian Industries, Limited, McMasterville, Quebec, carried out some work comparing the infrared spectra of cellulose, ω-cellulose, and carboxymethyl cellulose in an attempt to demonstrate compound formation. The results gave some indication that such was the case, but the experimental difficulties involved in these measurements made it impossible to draw any definite conclusions.

ω-Cellulose was diazotized and coupled by standard procedures to form colored azo derivatives. Using β-naphthol, the color of the product varied from a light pink, from ω-cellulose of D.S. 0.01 or less, to a very deep and brilliant red, from ω-cellulose of D.S. 0.20. At high magnification under a microscope the colors appeared to be uniform. Neither alkaline hypochlorite solution, 3% and 30% hydrogen peroxide, nor an acid solution of sulphur dioxide had any visible effect on the color of these derivatives.

An obvious possible application of ω-cellulose-azo compounds is their xanthation and spinning as molecularly-colored rayon. Viscose prepared from coupled ω-cellulose (D.S. 0.01) was spun into isotropic filaments by the method of Hermans (27). The thread, uniform, light red in color, could be stretched more than 100% and was of fair strength. Several yards of filament could be spun without a break, and under the microscope appeared smooth, uniform, and evenly colored. The same procedure was carried out with uncoupled ω-cellulose (D.S. 0.01). Almost colorless filaments were obtained, which could then be diazotized and coupled, or dyed directly with suitable acid dyes. A colored derivative prepared from ω-cellulose (D.S. 0.21) was also xanthated. The product dissolved only with difficulty in 6% sodium hydroxide solution and had to be diluted with more 6% sodium hydroxide solution before being filterable by suction. An attractive, bright-red filament was obtained by spinning the viscose solution as before. As mentioned previously, Industrial Cellulose Research, Limited, found that xanthated ω-cellulose of D.S. 0.33 was insoluble in sodium hydroxide solution. It thus appears that the solubility of the xanthate falls off sharply as the D.S. of ω-cellulose increases. It is possible, however, that this might be at least partially offset by increasing the D.S. of the xanthate groups. This point is being investigated.

It was found possible to render ω-cellulose-azo compounds water-soluble by carboxymethylation. The reaction was carried out on samples ranging in D.S. from 0.01 to 0.33. After purification by dialysis (34), the products were analyzed for D.S. of carboxymethyl group by the method of McLaughlin and Herbst (35). For ω-celluloses of D.S. 0.21 and 0.33, the D.S. of carboxymethyl groups had to be greater than 1.0 to make the product water-soluble. Degrees of substitution up to 1.7 were obtained. Whereas ordinary sodium carboxymethyl cellulose of a low D.S. is soluble in water, a considerably higher degree of substitution seemed to be required to confer complete solubility on the sodium carboxymethyl cellulose prepared from colored ω-cellulose.

Direct dyeing of cellulose is difficult because, unlike wool and silk, it contains no strongly polar groups such as the amino or carboxyl. ω-Cellulose, on the other hand, should possess direct-dyeing properties. A sample of ω-cellulose (D.S. 0.21) was dyed with a number of acid dyes such as croceine scarlet 3BX, croceine orange G, fast light green, and intensive blue. The dyeing procedures were adapted from those of Cain and Thorpe (4) and yielded attractive, intense, uniform colors which were fairly fast to washing. Controls run with shredded cellulose and absorbent cotton gave products from which the dyes were readily and completely removed by washing.

Nitration of ω-cellulose using standard techniques produced a material which was soluble in acetone. On evaporation of the filtered acetone solution a thin, opaque, yellow-brown, brittle film was obtained.

Samples of rayon yarn, paper towelling, surgical gauze, and factory cotton were soaked in 18% sodium hydroxide solution, pressed, washed with ethanol, and subjected to the usual etherification procedure. The D.S. of the samples were: ω-rayon, 0.01; ω-paper, 0.08; ω-gauze, 0.01; ω-cotton, 0.02. Diazotizing

and coupling with β-naphthol gave bright colored materials. The paper was red, the others orange. Direct dyeing with acid dyes was also effective, as before. Tensile strength tests on the rayon by Industrial Cellulose Research, Limited, revealed that, as expected, the tensile strength had been reduced by more than half. However, if the rayon derivative were required this effect can be avoided by etherifying before xanthation and regeneration. The low D.S. shows that the etherification conditions would need to be altered if the high D.S. obtained with shredded cellulose were desired, though intense, uniform colors are produced even with the low D.S. reported.

EXPERIMENTAL

Materials and Methods

For preparing alkali cellulose and for carrying out some of the reactions a Baker-Perkins Model No. 4–AN2 Werner-Pfleiderer shredder was used. A very much smaller model of this machine, having inside dimensions 2 × 2 × 4 in. (1/10th volume), was made later when some of the desired reagents proved to be difficult and expensive to make in the quantities required by the larger machine.

The only practical method of purifying the reaction products was by extraction of the unused reagents and by-products with suitable solvents. The extent of extraction necessary could often be judged by color since most of the reagents and their products of hydrolysis yield colored extracts. Therefore, when several consecutive extracts were colorless, purification was considered complete. In doubtful cases this was confirmed by establishing the constancy of the D.S. during the later stages.

The most convenient method for determining the extent of etherification was to analyze the products for nitrogen introduced as amino or nitro groups. The semimicro-Kjeldahl apparatus and procedure of Redemann (44) was used, with minor modifications. This involved 50 mgm. samples; reduction of any nitro groups with dextrose, according to Elek and Sobotka (14); selenium as a catalyst for digestion; collection of the ammonia evolved in 4% boric acid solution, and titration with 0.02 *N* hydrochloric acid, using a mixed indicator of methyl red and bromocresol green (31).

The *p*-amino-ω-chloroacetophenone used in this work was prepared by the method of Kunckell (32). Acetanilide was subjected to a Friedel and Crafts reaction with chloroacetyl chloride using aluminum chloride as a catalyst. The acetyl group was then removed by hydrolysis with aqueous hydrochloric acid. Pale, lemon-yellow crystals were obtained after repeated recrystallization from ethanol.

p-Nitrobenzyl chloromethyl ketone is a new compound. It was prepared by the chloromethylation of *p*-nitrophenylacetyl chloride after the method of McPhee and Klingsberg (36). Faintly yellow needles were obtained after recrystallization from ethanol (m.p. 94.0–94.5°C.; % nitrogen: calc. for $C_9H_8O_3NCl$: 6.56%; found: 6.59%).

ω-(p-Aminoacetophenone) Ether of Cellulose

Preparation

Ten grams of dry, shredded cellulose were steeped in 20% sodium hydroxide solution, pressed to a press-factor of three, slurried with just enough 95% ethanol to cover the mass, allowed to stand for one minute, and filtered. The ethanol washing was repeated as often as required (usually twice) (see Fig. 1). The product was refluxed for eight hours with 400 cc. of ethanol containing 15 gm. of *p*-amino-ω-chloroacetophenone. The light-yellow fibrous product was filtered, washed with hot acetone, water, and finally with acetone until the extract was colorless.

Hydrolysis with 72% Sulphuric Acid

Hydrolysis by sulphuric acid (37, 52, 53) was carried out as follows: Eight grams of ω-cellulose (D.S. 0.20) was covered with 100 cc. of cold 72% sulphuric acid, and the mixture, which soon became very dark colored, was allowed to stand at room temperature for 18 hr. The mixture was added to 3400 cc. of distilled water and refluxed for five hours. The dark-brown flocculent precipitate that separated on cooling was filtered off, and the red filtrate was neutralized with a hot aqueous solution of barium hydroxide. After removal of the barium sulphate, the yellow filtrate was concentrated to 175 cc. by evaporation at 50 mm. and 50°C. The concentrate was dialyzed through cellophane against three 225 cc. portions of distilled water and the combined dialyzates were reduced in volume to 150 cc. Unsubstituted glucose was removed from the concentrate by fermentation according to the method of Chen *et al.* (5). After the resulting mixture was centrifuged and filtered, the filtrate was evaporated to dryness, yielding a dark-red sirup. This was extracted with 30 cc. of dry, acetone-free methanol and treated with activated carbon to give a dark-red extract. A fraction obtained from this extract by chromatography on a Magnesol: Supercel column (2) (this column freely passed glucose and *p*-amino-ω-hydroxyacetophenone) gave a positive test for primary aromatic amino groups and a positive Molisch's test for carbohydrates. The nitrogen content (1.94%) was, however, too low for ω-glucose (4.47%).

Diazotization and Coupling

Ten grams of ω-cellulose suspended in 300 cc. of water was cooled to 5°C., 50 cc. of 1:1 hydrochloric acid was added, and the mixture cooled to 0°C. Two grams of sodium nitrite in 20 cc. of water was added, and the whole was allowed to stand for five minutes. The diazotized ω-cellulose was filtered off and washed with ice water. It was then added to a cooled solution (5°C.) made by dissolving 7.5 gm. of β-naphthol in 50 cc. of ethanol and adding 30 cc. of 20% sodium hydroxide solution and 300 cc. water. The mixture was vigorously stirred and then allowed to warm to room temperature and stand for half an hour. The colored ω-cellulose was filtered and thoroughly washed with water and ethanol.

Xanthation and Spinning

Five grams of ω-cellulose (D.S. 0.01) diazotized and coupled with β-naphthol was treated as in making alkali cellulose and then xanthated with 1.66 cc.

carbon bisulphide for 2.5 hr. in a small shredder. After the resulting 14 gm. of the red xanthate was added to 40 cc. of 6% sodium hydroxide solution, the mixture was kneaded for one hour. The resulting viscose solution was filtered without much difficulty through a thick, cotton pad and yielded a filtrate containing no undissolved fibers visible under the microscope. Isotropic filaments were spun by the method of Hermans (27) by forcing the viscose solution by low pressure through a fine glass capillary below the surface of a 14% ammonium sulphate solution.

Carboxymethylation

Five grams of ω-cellulose (D.S. 0.21) coupled with β-naphthol was mixed in the small shredder for 30 min. with 1.25 gm. of sodium hydroxide in 6.5 cc. of water; 3.5 gm. of powdered sodium chloroacetate was added, the mixture was shredded for 2.5 hr. and allowed to stand overnight; 1.25 gm. of flake sodium hydroxide was added and shredding was carried out for 30 min.; 3.5 gm. of sodium chloroacetate was added, shredding was continued for two hours, and the mixture was allowed to stand overnight. A sample was removed for analysis, and a third carboxymethylation step was carried out. The sticky, dark-red product was removed from the shredder and dissolved in water. The solution was purified by dialysis, using a cellophane membrane (34).

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A 27°C. ISOTHERMAL CALORIMETER[1]

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ABSTRACT

The need for a Bunsen-type isothermal calorimeter operating as close as possible to the standard thermochemical reference temperature, 25°C., was fulfilled by means of diphenyl ether (m.p. 26.9°C.) as the working substance. The instrument was calibrated electrically by comparison with the ice calorimeter and the constant was found to be 19.01±0.02 cal. per gm. of mercury. From this and other known properties of diphenyl ether the density of the solid at the melting point was estimated at 1.188 gm. per ml. Check determinations based on the heat of vaporization of water showed that for such measurements the mantle of solid ether must first be melted inside by an amount equivalent to the heat to be measured. The calorimeter was operated in a large water-thermostat kept constant to within 0.001°C. With highly purified diphenyl ether there was no noticeable temperature drift during the measurements. Quantities of heat up to 600 cal. could be measured with a reproducibility of the order of 0.2%. The new calorimeter is simpler to operate than the ice calorimeter and its sensitivity is more than three times as great.

INTRODUCTION

As part of a research program on hydrogen and deuterium peroxides (9) it was required to measure some thermochemical properties of these compounds such as the heats of vaporization, heats of solution, and heats of decomposition. Preliminary measurements of these quantities made in this laboratory with the ice calorimeter were reported a few years ago (5). Although generally satisfactory for this kind of work the ice calorimeter suffers from certain drawbacks such as low sensitivity and tedious operation. Furthermore, it is necessary to correct the results to 25°C., the standard temperature for thermochemical data. To minimize the uncertainty of such corrections it would be desirable to work as near as possible to that temperature. A number of Bunsen-type calorimeters operating with various organic compounds have been described in the literature. Coffin (1, 2) has reviewed the question and discussed the requirements for a suitable working substance. Diphenyl methane, m.p. 24.5°C. (11), and diphenyl ether, m.p. 26.9°C. (8, 10), have been used in the past for that purpose. On the basis of availability and stability towards oxidation the second was selected as more promising, a choice vindicated by recent investigations at the National Bureau of Standards (6).

In fact three or four distillations of a commercial product, followed by an equal number of crystallizations, were sufficient to obtain the ether in such a degree of purity that no "premelting" could be detected during the measurements. Another convenient feature of the diphenyl ether calorimeter is that it operates so near the average temperature of the laboratory that all thermal leaks are reduced to a minimum and a simple water-thermostat provides an

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entirely satisfactory surrounding. After more than three years of trouble-free operation we are convinced that this simple isothermal calorimeter is ideally suited for the measurement of heat effects accompanying physical or chemical changes at ordinary temperature.

EXPERIMENTAL

Two slightly different calorimeters were built in the course of this investigation. The most successful one will be described here together with suggestions for future improvements.

Construction of the Calorimeter

The calorimeter itself was made entirely of Pyrex glass although the use of a metal tube with appropriate seal for the central well, as suggested by some authors, would no doubt improve the heat transfer to the mantle. The dimensions shown in Fig. 1 are considered optimum; they are by no means

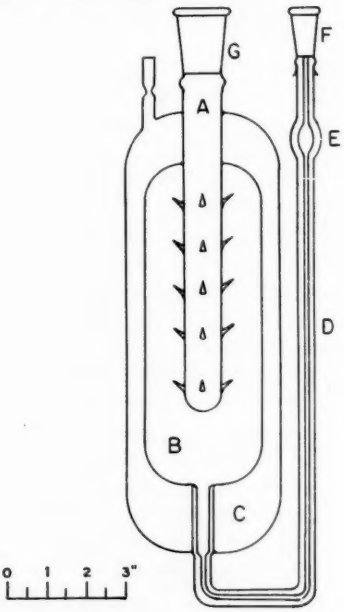

FIG. 1. Optimum dimensions for a diphenyl ether isothermal calorimeter of some 600 cal. capacity.

critical except, possibly, for the glass studs outside the central tube A which are needed to keep the mantle of frozen ether from slipping down. If made too short, they will not allow measurement of large enough amounts of heat; if too long, there is the danger of puncturing the mantle, with resulting heat loss, because the thermal conductivity of glass is appreciably higher than that of diphenyl ether. From the experience gained, 15 to 20 glass studs $\frac{3}{8}$ in. long would be adequate for a calorimeter of the size shown here.

The tube connecting the middle chamber B with the outside was first made S-shaped between the two jackets for greater elasticity. However, the inner seal soon broke merely on heating in an air-oven for drying. On the advice of a professional glass blower that section was replaced by a straight, thick-walled tube which, indeed, gave no trouble thereafter. The small bulb E, $\frac{1}{2}$ in. diameter blown on the capillary tube near the top, was found useful for trapping air bubbles before they reach the middle chamber. After cleaning thoroughly by the standard procedures the outside jacket C was evacuated down to 10^{-5} mm. Hg by means of a diffusion pump while the whole calorimeter was heated to about 100°C. in an oven. Just before sealing off the connecting tube a charcoal trap connected to the line was cooled in liquid air. In the first calorimeter the vacuum jacket had been silvered inside, except for a middle strip, but this was later found unnecessary. An outer shield of aluminum foil with opposite rectangular windows is sufficient to stop any radiant energy. It also affords a clearer view of the inside of the calorimeter, which is of great convenience, and it can be removed to speed up melting of the mantle when necessary.

Purification of the Diphenyl Ether

The starting product, Eastman's C.P., was first crystallized twice and then distilled four or five times under reduced pressure, 1.5 mm. Hg at about 102°C. Each time generous head- and tail-fractions were discarded until finally the distillation temperature remained constant to ±0.5°C. for the whole 800 ml. batch. To fill the calorimeter some 50 ml. of twice-distilled mercury were first introduced into chamber B. Then the calorimeter was mounted in an inverted, inclined position and the capillary tube was connected through the standard joint F to an "adapter tube" similar to those used in vacuum distillation apparatus. The lower end of that tube, reaching near the bottom of a 2-liter flask containing the pure ether, was bent so that it connected both the middle chamber of the calorimeter and the space above the ether to a vacuum pump and a cold trap. First the ether was rid of all dissolved gases by three successive freezings and meltings under reduced pressure. The solubility of air in diphenyl ether is so great that on the first freezing *in vacuo* the liquid seemed to boil violently. After three such operations no more dissolved gas was liberated. Then the bent tube was rotated by 180° about the ground joint and air was admitted above the liquid ether forcing it up into the calorimeter. A small residual bubble of vapor was expelled by blowing steam into the central well and then turning up the calorimeter while still heating it with steam until the mercury had filled the capillary tube up to the ground joint F.

Operation of the Calorimeter

The device for measuring the mercury was of the type developed for the ice calorimeter at the National Bureau of Standards (7). However, a steel needle-valve was not available so that an ordinary glass stopcock had to be used. This was somewhat unsatisfactory because only very little grease could be used on it and frequent cleaning was necessary. The calibrated capillary tube also required repeated cleaning as it was so fine (0.6 mm. diameter) that

mercury tended to stick in it. This part of the measuring device served only occasionally to obtain information on the thermal leak. Weighing of the displaced mercury in tiny beakers was done to the nearest 0.1 mgm. A mantle of solid ether was formed slowly by immersing a cold rod in the central well half-full of mineral oil for better heat transfer. In general one mantle could serve for two or three determinations. Before making a new mantle the old one was always melted completely. An infrared lamp was found very useful for this purpose. Apparently the manner of forming a mantle was of no great importance. Either ordinary ice or dry ice could be used, with rates varying from 20 min. to one hour. Usually it took five hours for a new mantle to come to equilibrium with the liquid ether.

The water-bath surrounding the calorimeter was a large aquarium made of plate-glass. Adequate temperature control was secured by means of a continuously adjustable "Magna-Set" mercury regulator and an electronic relay actuating a 500-watt immersion heater. A Cenco centrifugal stirrer provided efficient agitation. An auxiliary heater, hand-controlled through a Variac, was added for cold periods whereas a circulation of tap water was occasionally needed for operation in the summer. With such an arrangement the temperature could be kept constant indefinitely to within 0.03°C. but this was not quite sufficient. For a better temperature control and a more regular heat leak the principle of "internal insulation" suggested by Tian (12) was resorted to. The calorimeter proper was enclosed in a square box of Plexiglass which, effectively, provided an insulation of three inches of water between the calorimeter and the surrounding thermostat. As a result temperature fluctuations inside the box became smaller than 0.001°C. and the thermal leak of the calorimeter remained constant for a whole day instead of changing from hour to hour. What is important, indeed, is not so much the absolute value of this leak as its constancy, as has been pointed out by other investigators. As a matter of fact it was found preferable throughout this investigation to work with a steady, measurable thermal leak rather than with a vanishingly small one.

The temperature of the water-bath that gave the smallest leak (less than 0.2 cal. per hr.) was 26.90°C., as measured with a Leeds and Northrup certified platinum-resistance thermometer. In order to minimize fluctuations in the melting temperature of the diphenyl ether and, consequently, variations in the heat leak, the level of mercury in the measuring device was kept as constant as possible throughout a run. Another minor source of error stemmed from the small electric bulb used to light the calorimeter from behind. This energy amounted to only 0.3 cal. per hr. and could easily be accounted for.

Calibration of the Calorimeter

Usually such calibrations are carried out by absolute electric measurements. Since an ice calorimeter was available and since the factor of this instrument is known with great accuracy (7) it was decided to use it as reference for calibrating the diphenyl ether calorimeter. Two resistances R_1 and R_2 of about the same value were immersed in the central well of each calorimeter and connected in series. On passing an electric current in the circuit the amount

of heat dissipated in each calorimeter is obviously proportional to the values of R_1 and R_2. Therefore the electric energy need not be measured exactly; even the exact values of R_1 and R_2 are not required. Indeed, if a second series of experiments is made with the resistances interchanged then the calibration factors f of the two calorimeters are related thus,

$$f_e\sqrt{W_e \cdot W_e'} = f_i\sqrt{W_i \cdot W_i'},$$

where W and W' are the weights of mercury displaced in each set of determinations in each calorimeter.

The two resistances were made of 10-ohm windings of No. 34 Advance wire wound on thin glass tubes and connected to leads of No. 26 copper wire. In order to reduce the heat leak, particularly in the ice calorimeter, the leads were connected directly to "thermal shunts" made of sections of insulated brass foil immersed in the surrounding ice-bath. In spite of this precaution the leak of the ice calorimeter was nearly three times greater than normal which, in fact, represented the major uncertainty in these measurements. From the results collected in Table I and the factor for the ice calorimeter,

TABLE I

CALIBRATION OF THE DIPHENYL ETHER CALORIMETER BY COMPARISON WITH THE ICE CALORIMETER

Run. No.	Thermal leak (cal./hr.)				Approximate energy supplied (cal.)	W_e/W_i
	Ice calorimeter		Ether calorimeter			
	Initial	Final	Initial	Final		
First series						
1	1.27		1.43		430	3.0850
2	1.15	1.06	1.60	1.55	430	3.0890
3	1.41	1.48	2.39	2.45	280	3.0897
4	1.48		2.01		430	3.0881
5	1.13		0.35		200	3.0917
					Average	3.0887
Second series						
6	1.32	1.45	0.29	0.19	230	3.7434
7	1.34		0.24		150	3.7444
8	2.21		0.06		280	3.7431
9	2.05	1.99	0.44	0.31	380	3.7403
10	1.67		0.07		380	3.7422
11	2.22	1.76	0.38	0.13	400	3.7427
12	2.37	1.33	0.28	0.43	330	3.7450
13	2.08	1.37	1.37	1.70	380	3.7409
14	1.70	2.10	0.08	0.13	250	3.7405
15	1.84	3.51	0.28	0.19	320	3.7428
					Average	3.7425

$f_i = 64.640 \pm 0.007$ cal. per gm. of mercury (7), one finds for f_e 19.01 cal. per gm. of mercury with a standard deviation of 0.02 unit. Thus the diphenyl ether calorimeter has a sensitivity 3.4 times that of the Bunsen ice calorimeter. Holmberg (8) had obtained a slightly higher factor, 20.49, and Sachse (10),

a much greater one, 27.20 cal. per gm. of mercury, but the ether used by the latter must have been impure as shown by the melting point, 26.55°C. From the equation

$$f_e = L/\Delta v \,.\, d_m,$$

where L is the heat of fusion of the ether, 24.176 cal./gm. (6), and d_m is the density of mercury, we get 0.08574 for Δv, the change of specific volume of the ether on melting. The density of the liquid is 1.06611 at 30°C. and 1.06117 at 35° (4). Extrapolation to the melting point gives 1.0692 and from the above value of Δv the density of the solid at that temperature is calculated to be 1.188 while the coefficient dt/dp for the melting curve is 0.025° per atm. On the other hand, the triple point of diphenyl ether is known accurately: 26.87±0.01°C. from the measurements at the National Bureau of Standards. Therefore the melting point is 26.89$_5$°C. Adding to this the pressure effect of the mercury column above the mantle of solid ether (30 cm.) we arrive at the exact value 26.90° observed for the temperature of minimum leak, which confirms the high purity of the ether in the calorimeter. Dodd and Hu Pak Mi (3) found 26.85°C. by direct measurement and a slightly higher value, 26.95°C., from the break in the curve of viscosity vs. temperature.

A first series of calibrations of the new calorimeter based on the heat of vaporization of water had given a somewhat greater factor, 19.10 cal. per gm. of mercury. Since previous investigators have recommended that isothermal calorimeters be calibrated in the same way they are to be used, the cause of this discrepancy was investigated in order to apply it to measurements of the heat of vaporization of hydrogen and deuterium peroxides. For these check measurements a weighed 0.5 gm. sample of distilled, deaerated water in a thin glass tube was immersed in the calorimeter well and connected through a standard joint with a vacuum pump and a cold trap. The calibration factors thus obtained seemed to depend on the rate of evaporation of the water. As a high value of the factor indicates a loss of heat, it was suspected that the vapor left the calorimeter at a temperature lower than 26.9°C. No such difficulty had been encountered in similar measurements with the ice calorimeter so the explanation was thought to rest with the lower thermal conductivity of diphenyl ether. (No data are available on this compound in particular but organic liquids and solids generally have thermal conductivity about ten times smaller than that of ice.) To check this point a few determinations were carried out after first premelting the mantle of solid ether by an amount at least equal to the heat effect to be measured. The results in Table II seem to bear out the above contention, specially the first run where no premelting led to an abnormally high factor, and the second one, where only partial premelting gave an intermediate value. Still, the factors obtained by that method were always appreciably higher than by the more reliable electrical method, an indication that premelting does not provide exact compensation. At any rate the average value of f_e from these determinations, 19.04±0.04 cal. per gm. of mercury, was used only in measurements of heats of vaporization.

TABLE II

CALIBRATION OF DIPHENYL ETHER CALORIMETER BASED ON THE HEAT OF VAPORIZATION OF WATER

Run No.	Heat absorbed, cal.	Premelting, cal.	Thermal leak		Calibration factor, f_e
			Initial, cal./hr.	Final, cal./hr.	
20	345	Nil	0.213	0.217	19.17
21	135	90	0.308	0.308	19.08
22	223	250	0.275	0.216	19.07
23	283	320	0.217	0.192	19.07
24	312	320	1.18	0.776	18.99
25	264	400	0.125	0.038	19.08
26	246	380	1.15	0.845	19.04
27	321	330	0.61	0.55	19.03

CONCLUSION

The diphenyl ether isothermal calorimeter has proved to be a reliable and useful research instrument for measuring thermal phenomena at room temperature. It has the advantage of great simplicity of construction and operation. For over three years it has remained constantly in working condition with very little attention. Operation near room temperature reduces to a minimum, not only the thermal leaks, but also the correction of results to the accepted temperature for thermochemical data. The use of a water-thermostat means considerable simplification over the ice calorimeter. Diphenyl ether is currently available and easily purified; once in the calorimeter it seems indefinitely stable.

The aim of this investigation was to determine some calorimetric properties of hydrogen and deuterium peroxides (to be published in a following paper). Lack of time and equipment did not allow an exhaustive study of the possibilities of the diphenyl ether calorimeter. This is particularly true of the calibration, which should be repeated by an absolute electrical method to take full advantage of the greater sensitivity of the instrument.

RÉSUMÉ

On a mis au point un calorimètre isotherme du type de Bunsen fonctionnant avec l'oxyde de phényle dont le point de fusion est 26.9°C. Le facteur de calibration, 19.01 ± 0.02 cal./g. de mercure, a été déterminé électriquement par comparaison avec le calorimètre à glace. Le nouveau calorimètre est très simple de construction et de manipulation; il est contenu dans un thermostat à eau. Avec le modèle décrit on a pu mesurer jusqu'à 600 cal. avec une précision de l'ordre de 0.2%. Les fuites et corrections sont réduites au minimum parce que la température de fonctionnement est assez voisine de celle du laboratoire.

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REACTIONS BETWEEN SOLID CALCIUM CARBONATE AND ORTHOPHOSPHATE SOLUTIONS[1]

By J. S. Clark and R. C. Turner

ABSTRACT

Studies of the reactions between solid calcium carbonate and orthophosphate solutions at low partial pressures of carbon dioxide indicated that hydroxyapatite was the stable product formed in these systems. Hydroxyapatite precipitated directly even though dicalcium phosphate formed initially under certain conditions. The reactions with orthophosphate did not seriously disturb the normal calcium carbonate equilibrium with the solution.

INTRODUCTION

Owing to the importance of calcium carbonate in phosphate fixation (the conversion of soluble phosphates to less soluble forms) in calcareous soils, considerable attention has been given to reactions between phosphate in solution and solid calcium carbonate. Boischot *et al.* (2) and Cole *et al.* (4) showed that, at relatively low concentrations, phosphate was adsorbed on the surface of the calcium carbonate crystals. At higher concentrations there was apparently chemical precipitation of a calcium phosphate. Cole and his co-workers presented evidence that dicalcium phosphate was precipitated and they implied that this compound was an intermediate in the formation of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$).

The object of the work reported here was (*a*) to show the effect of the surface reaction between phosphate and calcium carbonate on the equilibrium between the solution and solid calcium carbonate, and (*b*) to obtain more information on the kind of reaction which takes place.

EXPERIMENTAL

Equilibration and rate studies of the reactions between solid calcium carbonate and solutions of phosphates at various initial concentrations were made at two partial pressures of carbon dioxide. The partial pressures of carbon dioxide in the systems were obtained by constantly bubbling through the suspensions either air piped in from just outside the laboratory window or a commercial mixture of 5% CO_2 and 95% N_2. The versene method (1) was used for the determination of calcium concentrations except in the rate studies where the flame photometer was found to be more convenient. The molybdenum blue method (5) was used for phosphorus and the glass electrode for pH. The activities of the ions were estimated by means of the Debye Huckel equation (7).

RESULTS

The results of the equilibration experiments are plotted as points in Fig. 1 in which the solubility lines are calculated from published data (6, 8). The

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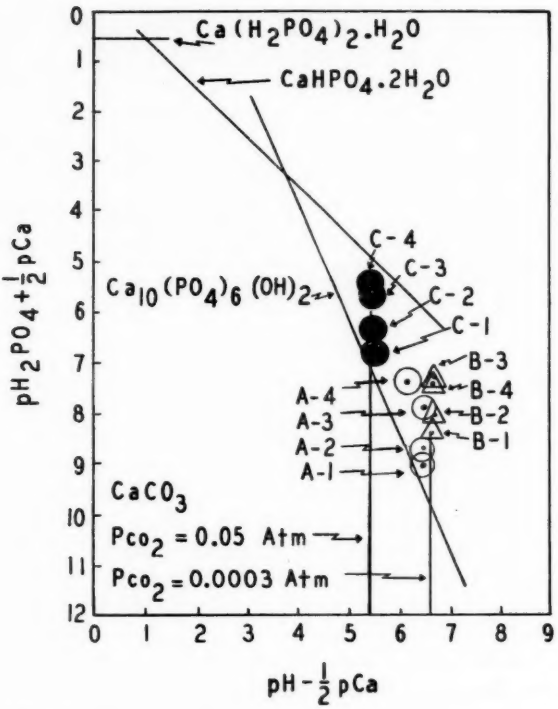

FIG. 1. Solubility diagram.

Legend:

P_{CO_2} = 0.0003 atm. Series A and B, 0.05 atm. Series C.

Initial phosphate concentrations:

A—1 10^{-6} *M* KH_2PO_4	B—1 5×10^{-6} *M* H_3PO_4	C—1 10^{-5} *M* H_3PO_4
A—2 5×10^{-6} *M* KH_2PO_4	B—2 10^{-5} *M* H_3PO_4	C—2 2×10^{-5} *M* H_3PO_4
A—3 10^{-5} *M* KH_2PO_4	B—3 5×10^{-5} *M* H_3PO_4	C—3 10^{-4} *M* H_3PO_4
A—4 10^{-4} *M* KH_2PO_4	B—4 10^{-4} *M* H_3PO_4	C—4 2×10^{-4} *M* H_3PO_4

solubility diagram is discussed fully elsewhere (3); however, a brief explanation of the diagram is included in the appendix.

After the 5% CO_2 and 95% N_2 mixture was bubbled through the suspensions for two weeks, the values of $pH - \frac{1}{2}pCa$, regardless of the initial concentration of phosphate, were reasonably close to the line in the diagram for calcium carbonate at P_{CO_2} (partial pressure of carbon dioxide) equal to 0.05 atmosphere. When air was bubbled through the suspensions for two to five weeks, the average value of $pH - \frac{1}{2}pCa$ was 6.5 compared with a calculated value of 6.6 for $P_{CO_2} = 0.0003$ atmosphere which is the average partial pressure of CO_2 in the air. There was more variation within a series when air was bubbled through the suspensions but this is probably the result of variations in the carbon dioxide content of the air. As the solubility product of $CaCO_3$ was generally satisfied, it seems unlikely that contamination from the atmosphere, e.g. NH_3 or SO_2, was serious.

The experimental data plotted on Fig. 1 show that although the solutions were virtually at equilibrium with solid calcium carbonate, they were not at equilibrium with a calcium phosphate compound. The fact that the experimental points are above the hydroxyapatite line indicates that equilibrium was reached slowly in these systems; therefore experiments were made to study the progress of the reactions towards equilibrium.

In Table I are presented results of experiments in which samples were removed at intervals while the carbon dioxide – nitrogen mixture was bubbled

TABLE I

EFFECT OF TIME ON REACTION AT P_{CO_2} APPROXIMATELY 0.05 ATMOSPHERE

Reaction time, min.	pH	$pH-\frac{1}{2}pCa$	$pH_2PO_4+\frac{1}{2}pCa$	Total P, moles/l. $\times 10^3$	$a_{Ca^{++}} \cdot a_{HPO_4} = \times 10^{7*}$
(*a*) Initial solution approximately 2.5×10^{-2} *M* H_3PO_4					
20	5.56	4.42	2.86	22.50	22.90
100	5.90	4.55	3.55	7.30	6.31
225	6.61	5.21	4.44	1.40	3.71
460	6.70	5.31	4.60	0.97	3.24
1440	6.69	5.32	4.63	0.89	3.09
1860	6.79	5.32	4.63	0.89	3.09
(*b*) Initial solution approximately 2.2×10^{-3} *M* H_3PO_4					
20	6.60	5.26	4.18	2.13	7.59
390	6.66	5.32	4.25	1.85	7.42
1440	6.56	5.20	4.47	1.10	3.39
1860	6.56	5.28	4.71	0.68	2.34
2820	6.59	5.21	5.10	0.28	0.83
3120	6.70	5.32	5.12	0.28	1.00
(*c*) Initial solution approximately 1.7×10^{-3} *M* KH_2PO_4					
65	6.73	5.38	4.33	1.64	7.08
240	6.64	5.20	4.56	1.11	2.75
1860	6.70	5.36	4.72	0.66	2.75
4440	6.76	5.37	4.95	0.44	1.66
11280	6.86	5.47	5.17	0.31	1.26
(*d*) Initial solution approximately 2.4×10^{-4} *M* H_3PO_4					
70	6.76	5.41	5.21	0.23	1.00
260	6.82	5.46	5.21	0.23	1.12
500	6.84	5.31	5.21	0.23	0.80
1440	6.76	5.42	5.29	0.18	0.85
6240	6.84	5.50	5.48	0.13	0.66

**a designates the ion activity. Solubility product of dicalcium phosphate is 2.19×10^{-7}.*

through phosphated suspensions of calcium carbonate. In each experiment 20 gm. of $CaCO_3$ was mixed with slightly less than 1 liter of water for 24 hr. by bubbling the gas through the suspensions. A sufficient volume of relatively concentrated phosphate solution was added to give the desired initial concentration and the solution was made up to 1 liter with water. Samples were taken at frequent intervals but only sufficient data to show the progress of the reactions are included in Table I.

It is seen from the second column of Table I that the solution was close to equilibrium with calcium carbonate within 20 min. except at the highest initial concentration of phosphate. With the highest concentration of phosphate, 2.5×10^{-2} *M* H_3PO_4, the values of $pH-\frac{1}{2}pCa$ did not approach that

required for P_{CO_2} equal to 0.05 atmosphere until after about four hours. There was a rapid initial precipitation of phosphate in this system. During the process of rapid precipitation the solution was supersaturated with respect to dicalcium phosphate. This can be seen by plotting the values of $pH_2PO_4 + \frac{1}{2}pCa$ and $pH - \frac{1}{2}pCa$ on the solubility diagram of Fig. 1 or more conveniently by comparing the values of $a_{Ca^{++}}.a_{HPO_4^=}$ in the last column of Table I with the solubility product of dicalcium phosphate. After the rapid precipitation, the phosphate concentration gradually reached a constant value for the remainder of the experiment and the solution was apparently at virtual equilibrium with dicalcium phosphate. With the intermediate initial concentrations of phosphate, 2.2×10^{-3} *M* H_3PO_4 and 1.7×10^{-3} *M* KH_2PO_4, the early rapid decrease in phosphate concentration was followed by a slower prolonged precipitation. Again the rapid precipitation occurred while the solution was supersaturated with respect to dicalcium phosphate, but the solution was definitely not supersaturated with respect to this solid during the slower precipitation. With the lowest initial concentration of phosphate, 2.4×10^{-4} *M* H_3PO_4, the solution was never supersaturated with respect to dicalcium phosphate and there was only a slow rate of decrease of the phosphate concentration during the whole experiment.

In Table II are reported data for experiments in which air was bubbled through the suspensions. For these experiments calcium carbonate was added directly to solutions of the various phosphates. When the phosphate solution

TABLE II

EFFECT OF TIME ON REACTION AT P_{CO_2} APPROXIMATELY 0.0003 ATMOSPHERE

Reaction time, min.	pH	$pH - \frac{1}{2}pCa$	$pH_2PO_4 + \frac{1}{2}pCa$	Total *P*, moles/l. $\times 10^3$	$a_{Ca^{++}}.a_{HPO_4^=} \times 10^{7*}$
(*a*) Initial solution approximately 1.56×10^{-3} *M* H_3PO_4					
20	7.40	5.95	4.79	1.50	9.13
65	7.56	6.02	5.10	1.10	5.25
100	7.80	6.33	6.19	0.17	0.87
360	8.00	6.38	6.57	0.10	0.41
4320	8.08	6.45	7.19	0.03	0.12
(*b*) Initial solution approximately 1.02×10^{-3} *M* KH_2PO_4					
50	7.80	6.16	5.57	0.72	2.46
120	8.04	6.30	6.13	0.33	0.93
300	8.15	6.47	6.67	0.12	0.40
4560	8.23	6.51	7.02	0.068	0.20
7200	8.25	6.53	7.11	0.058	0.17
(*c*) Initial solution approximately 1.01×10^{-3} *M* K_2HPO_4					
5	8.37	6.66	6.03	0.90	2.69
80	7.97	6.29	5.62	0.93	2.95
150	8.00	6.36	5.64	0.85	3.31
470	8.30	6.50	6.64	0.20	0.46
1380	8.30	6.47	6.81	0.16	0.29
(*d*) Initial solution approximately 0.5×10^{-3} *M* K_2HPO_4					
5	8.85	6.50	6.91	0.47	0.25
90	8.30	6.49	6.20	0.48	1.23
360	8.12	6.40	6.08	0.47	1.32
2880	8.31	6.52	7.08	0.081	0.17
7200	8.42	6.61	7.44	0.048	0.09

**a designates the activity of the ion. Solubility product of dicalcium phosphate is 2.19×10^{-7}.*

was either H_3PO_4 or KH_2PO_4, about two hours were required for the solution to approach equilibrium with solid calcium carbonate. With K_2HPO_4 solutions, the values of $pH-\frac{1}{2}pCa$ were always reasonably close to the equilibrium value. The values of $pH-\frac{1}{2}pCa$, however, decreased gradually during the early part of these experiments but this was followed by a slow increase. The behavior of the systems in which the initial phosphate concentrations were 1.56×10^{-3} M H_3PO_4 and 1.02×10^{-3} M KH_2PO_4 was similar to that of the intermediate concentrations reported in Table I; the values of $a_{Ca^{++}}.a_{HPO_4^{=}}$, however, decreased more rapidly at P_{CO_2} equal to 0.0003 atmosphere. When the initial phosphate concentration was 1.01×10^{-3} M K_2HPO_4 there was apparently no reaction for the first two hours even though the solution was supersaturated with respect to dicalcium phosphate. After two hours there was a rapid decrease in the phosphate concentration followed by a slower reaction. With the lowest phosphate concentration, 0.5×10^{-3} M K_2HPO_4, the value of $a_{Ca^{++}}.a_{HPO_4^{=}}$ never exceeded that for dicalcium phosphate and there was only a slow precipitation, which began after about six hours.

The rate of decrease of the phosphate concentration in the rate experiments reported in Tables I and II was extremely slow after the first rapid reaction. A long reaction period would be required for the solutions to reach equilibrium with hydroxyapatite. There are two possible reasons for this apparently slow reaction: one, that the solution was approaching equilibrium with some calcium phosphate other than hydroxyapatite; two, that it was simply a rate process, the rate being highly dependent on the phosphate concentration. The results in Fig. 2 suggest that the latter possibility is more probably correct

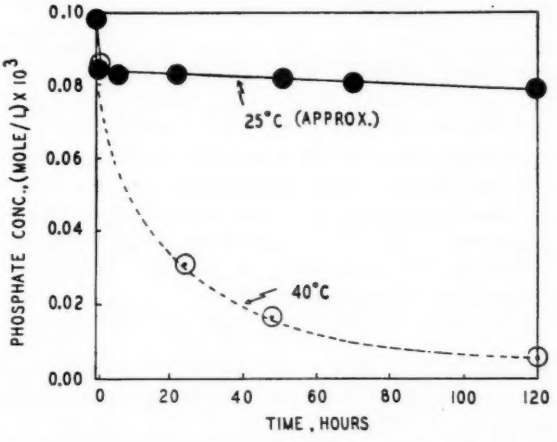

FIG. 2. Effect of temperature on rate of reaction at P_{CO_2} approximately 0.0003 atmosphere.

because the rate of decrease of the phosphate concentration was faster at 40°C. than at room temperature (about 25°C.) when the initial phosphate concentration was 0.098×10^{-3} M K_2HPO_4. The rate of decrease of the phosphate concentration at 40°C. was very slow after five days and, even

though the phosphate concentration was about one-tenth that of the lowest concentration obtained at room temperature, the solution was still supersaturated with respect to hydroxyapatite. In an experiment at 80°C., however, the solution was close to equilibrium with hydroxyapatite within 24 hr.

DISCUSSION

Regardless of the mechanism of the reaction between solid calcium carbonate and dilute solutions of phosphate, the calcium carbonate exerts its normal effect on the solution equilibrium.

It is seen from the diagram of Fig. 1 that, under the conditions prevailing in these experiments, dicalcium phosphate is not thermodynamically stable. As it is generally agreed that tricalcium phosphate does not exist in nature, the thermodynamically stable form is probably hydroxyapatite. If the solution is supersaturated with respect to dicalcium phosphate, however, this compound precipitates because hydroxyapatite forms at a much slower rate. This conclusion is based on the results reported under (*a*) in Table I which shows that about 21×10^{-3} mole of phosphorus was precipitated in less than eight hours, yet the solution was apparently supersaturated with respect to dicalcium phosphate after 31 hr. When the initial concentrations of phosphate were intermediate, (*b*) and (*c*) in Tables I and II, dicalcium phosphate was apparently precipitated in small quantities. The small quantities remained only for a short time, the length of time apparently depending on the quantity present and the rate of precipitation of hydroxyapatite. Judging by the results under (*d*) of Tables I and II, there seems little doubt that hydroxyapatite precipitated directly without the necessity of having to form dicalcium phosphate as an intermediate.

It was mentioned above that the values of the product $a_{Ca^{++}}.a_{HPO_4^=}$ decreased more rapidly when air was bubbled through the suspensions than when the 5% carbon dioxide mixture was used, indicating a more rapid approach to equilibrium in the former case. A possible explanation of this is that the rate of precipitation of hydroxyapatite is dependent on the concentration of $PO_4^{\equiv}$ ions which would be lower in the more acid suspensions through which the CO_2–N_2 mixture was passed. This observation along with the resulting increase in the rate of phosphate precipitation with increasing temperature indicates the advisability of making a thorough study of the kinetics of precipitation of hydroxyapatite.

APPENDIX

The solubility diagram of Fig. 1 is based on the fact that the equilibrium formation of a number of solid calcium orthophosphates in aqueous systems may be expressed by the equation

$$m\mathrm{Ca(OH)_2}+n\mathrm{Ca(H_2PO_4)_2} = \text{solid}+y\mathrm{H_2O} \qquad [1]$$

where m and n represent the number of moles of $Ca(OH)_2$ and $Ca(H_2PO_4)_2$, respectively, forming 1 mole of solid and y moles of H_2O. In relatively dilute solutions, $Ca(OH)_2$ and $Ca(H_2PO_4)_2$ may be assumed to be completely dis-

sociated. It follows, therefore, that

$$-m(\mathrm{pH}-\tfrac{1}{2}\mathrm{pCa})+n(\mathrm{pH_2PO_4}+\tfrac{1}{2}\mathrm{pCa}) = k \qquad [2]$$

where "p" represents the negative logarithm of the ion activity, k is a constant, and the activity of OH^- ions is expressed in terms of the ionic product of water, K_W, and the H^+ ion activity. With $(\mathrm{pH}-\frac{1}{2}\mathrm{pCa})$ and $(\mathrm{pH_2PO_4}+\frac{1}{2}\mathrm{pCa})$ as the two co-ordinates, equation 2 represents a straight line with slope m/n.

The constant k in equation 2 can be evaluated from the solubility product of the solid, the second and third dissociation constants of phosphoric acid, K_2 and K_3, and the ionic product of water. For hydroxyapatite, monocalcium phosphate, and dicalcium phosphate equation 2 becomes, respectively, equations 3, 4, and 5 below,

$$-7(\mathrm{pH}-\tfrac{1}{2}\mathrm{pCa})+3(\mathrm{pH_2PO_4}+\tfrac{1}{2}\mathrm{pCa}) = \tfrac{1}{2}(\mathrm{p}K_{sp}{}^{\mathrm{I}}-6\mathrm{p}K_2K_3-2\mathrm{p}K_W) \qquad [3]$$

$$(\mathrm{pH_2PO_4}+\tfrac{1}{2}\mathrm{pCa}) = \tfrac{1}{2}\mathrm{p}K_{sp}{}^{\mathrm{II}} \qquad [4]$$

$$-\tfrac{1}{2}(\mathrm{pH}-\tfrac{1}{2}\mathrm{pCa})+\tfrac{1}{2}(\mathrm{pH_2PO_4}+\tfrac{1}{2}\mathrm{pCa}) = \tfrac{1}{2}(\mathrm{p}K_{sp}{}^{\mathrm{III}}-\mathrm{p}K_2) \qquad [5]$$

where $K_{sp}{}^{\mathrm{I}}$, $K_{sp}{}^{\mathrm{II}}$, and $K_{sp}{}^{\mathrm{III}}$ are the solubility products of the appropriate solid phases. The solubility lines in Fig. 1 were drawn from equations 3, 4, and 5 using published values (8) for the right-hand side of the equation. In the figure, the co-ordinates are arranged so that the chemical potential of $Ca(H_2PO_4)_2$ increases upwards and that of $Ca(OH)_2$ to the right. If the solution is in equilibrium with a given solid phase, the solubility points must lie along the solubility line for that compound. A solubility point to the right or above a solubility line indicates supersaturation or the existence of a metastable solid phase.

If the solution is at equilibrium with solid calcium carbonate, it can be shown that

$$-(\mathrm{pH}-\tfrac{1}{2}\mathrm{pCa}) = \tfrac{1}{2}(\mathrm{p}K_{sp}{}^{\mathrm{IV}}-\mathrm{p}K_1{}^{\mathrm{I}}K_2{}^{\mathrm{I}}-\mathrm{pC}-\mathrm{p}P_{CO_2})$$

where $K_{sp}{}^{\mathrm{IV}}$ is the solubility product of $CaCO_3$, $K_1{}^{\mathrm{I}}$ and $K_2{}^{\mathrm{I}}$ are the first and second dissociation constants of carbonic acid, P_{CO_2} is the partial pressure of CO_2 in the system, and C is the total molal concentration of CO_2 in solution when P_{CO_2} is unity.

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THE TERTIARYBUTYLBENZENES

I. ALKYLATION OF 1,4-DI-*t*-BUTYLBENZENE WITH *t*-BUTYL CHLORIDE[1]

By L. Ross C. Barclay and Eileen E. Betts[2]

ABSTRACT

The alkylation of *p*-di-*t*-butylbenzene with excess *t*-butyl chloride in the presence of aluminum chloride in the cold produces a new aromatic hydrocarbon, m.p. 218.5–219°. Evidence is given for the presence of an alicyclic nucleus in this hydrocarbon which analyzes for $C_{22}H_{34}$. The preparation of nitro and amino derivatives from this hydrocarbon is described. The alkylation of benzene under similar conditions yielded some 1,3,5-tri-*t*-butylbenzene, and an improved method of preparation of this hydrocarbon from *p*-di-*t*-butylbenzene is given.

INTRODUCTION

For some time we have been interested in synthesizing 1,3,5-tri-*t*-butylbenzene and in preparing derivatives of this compound required in a study of the influence of the bulky *t*-butyl group on functional group reactions. Recently Bartlett (1) showed that the reaction of *t*-butyl chloride and aluminum chloride with *p*-di-*t*-butylbenzene yields *m*-di-*t*-butylbenzene, 1,3,5-tri-*t*-butylbenzene, and a hydrocarbon of melting point 209–210°. This excellent communication prompted us to publish some of the work conducted in our laboratory on *t*-butylbenzenes.

We found that not only *p*-di-*t*-butylbenzene but also benzene is converted to a mixture of 1,3,5-tri-*t*-butylbenzene and a hydrocarbon of melting point 218–219°, when treated with excess *t*-butyl chloride and aluminum chloride. Bartlett's preparation of 1,3,5-tri-*t*-butylbenzene could not be repeated but an improved method is given in the experimental section (Run II). In these alkylations, 1,3,5-tri-*t*-butylbenzene is formed rapidly, but under the influence of excess aluminum chloride and prolonged reaction time it is converted to the higher melting hydrocarbon. Some interesting properties of this hydrocarbon are reported.

A New High Melting Hydrocarbon and Some Derivatives

A high melting (218.5–219°) aromatic hydrocarbon which analyzed for $C_{22}H_{34}$ was isolated by adding aluminum chloride in portions to a cold (below −5°) solution of *p*-di-*t*-butylbenzene in excess *t*-butyl chloride. This compound was nitrated and the nitro compound reduced to an amine. Attempts to convert the amine into a phenol whose properties could be compared with those of the known 2,4,6-tri-*t*-butylphenol (12) produced a pale yellow compound possessing an ultraviolet absorption intensity much higher than that of phenols. The properties of these derivatives indicate that they are highly hindered. For example, the nitro compound does not show the large increase in absorption in the near ultraviolet characteristic of nitro derivatives of

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Contribution from the Department of Chemistry, Mount Allison University, Sackville, New Brunswick.
[2]*Recipient of a National Research Council Bursary.*

benzene and in this respect is similar to the sterically hindered nitro compounds studied by Brown and Reagan (4). In addition, the amine derivative does not dissolve in concentrated acid.

Certain general conclusions can be made concerning the structure of the high melting hydrocarbon from the ultimate and spectral analyses. The higher carbon values (or lower hydrogen values) of this compound compared to those of the butylbenzenes suggest a second ring fused onto the benzene ring in the alkylation. This might also explain the high melting point. The infrared spectrum (Fig. 1) shows the presence of *t*-butyl groups. The most characteristic feature is the strong band at 891 $cm.^{-1}$ and the absence of any strong band below this. In this respect it resembles the spectrum of pentamethylbenzene, which may indicate that the hydrocarbon contains a penta-substituted ring. The ultraviolet absorption of this hydrocarbon is very interesting in that it shows similarities to that of certain alicyclics, for example indan. The latter shows characteristic bands at 273.6, 267, and 260 mμ compared to 278, 271, and an inflection at 262 mμ for this hydrocarbon. In addition the very weak band for indan at 291 suggested by Morton and de Gouveia (9) as being due to impurities is present in this compound at 298 mμ.

It should be noted that tetralin also has similar absorption with bands at 274 and 267 and an inflection at 261 mμ but the weak band near 290 mμ is not reported (9). It is possible that this weak band may be of use in identifying the indan nucleus in the high melting hydrocarbon.

Early Research on the Poly-t-butylbenzenes

In 1890 Senkowski (10) reported tri-*t*-butylbenzene, m.p. 128°, along with *p*-di-*t*-butylbenzene, m.p. 70°, and a liquid hydrocarbon from the alkylation of benzene with isobutyl chloride. Smith (11) obtained some of Senkowski's hydrocarbon, m.p. 128°, by the rearrangement of isobutylphenyl ether in benzene solution with aluminum chloride.

In a study of various complexes formed by the Friedel-Crafts reaction, Gustavson (5) found that the action of *t*-butyl chloride and benzene on aluminum chloride at −10° led to the formation of a yellow crystalline compound, aluminum chloride ditributylbenzene hydrochloride, $Al_2Cl_6[C_6H_3[(CH_3)_3C]_3]HCl$, which was also formed by the reaction of *p*-di-*t*-butylbenzene and powdered aluminum chloride with *t*-butyl chloride.

Ipatieff (6) and co-workers reported mono-, di-, and tri-*t*-butylbenzene (a liquid) from the reaction of isobutylene with a mixture of sulphuric acid and benzene. Koch and Steinbrink (7) alkylated benzene by the method described by Ipatieff and co-workers and obtained *p*-di-*t*-butylbenzene but were unable to prepare a tri-substituted product by this method. Legge (8) attempted to prepare tri-*t*-butylbenzene by the reaction of isobutylene with aluminum chloride or sulphuric acid on benzene but obtained instead a considerable number of unexpected alkylbenzenes and suggested an electronic explanation for the results obtained.

Bartlett and co-workers (1) found small quantities of Senkowski's hydrocarbon (m.p. 129.5–130°) in some residues from the preparation of *p*-di-*t*-

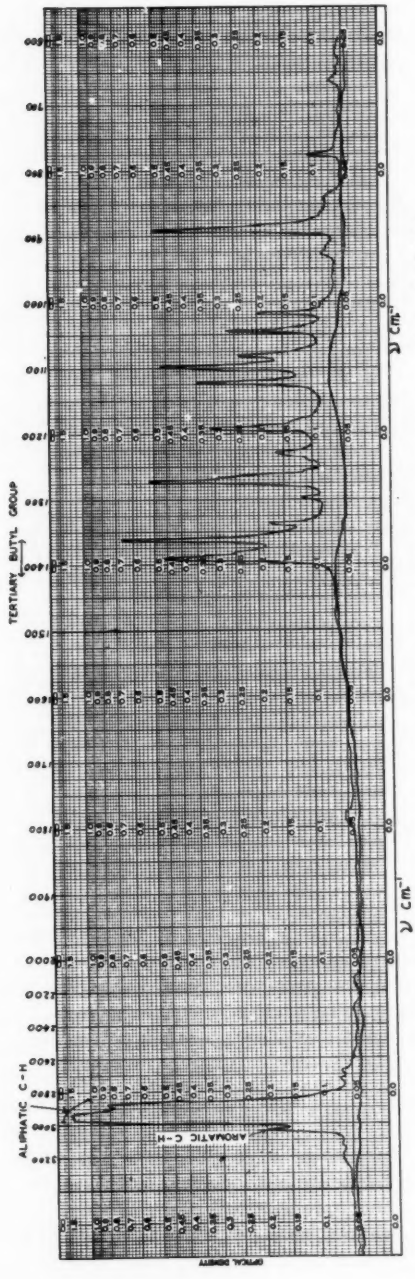

FIG. 1. Infrared spectrum of the high melting (218.5–219°) hydrocarbon in carbon disulphide.

butylbenzene by the Standard Oil Company of Indiana. However, these workers did not establish the structure of this hydrocarbon. It seems unlikely that the structure given to this compound by Smith (11), that is 1,2,4-tri-*t*-butylbenzene, is correct because of the improbability that two *t*-butyl groups can occupy adjacent positions in the benzene ring. Brown and co-workers (3) investigated homomorphs of *o*-di-*t*-butylbenzene and support this view.

EXPERIMENTAL

The melting points recorded were obtained with a Fisher-Johns type apparatus and are uncorrected. Ultraviolet spectra were measured on a Beckmann DU spectrophotometer with cyclohexane, free from aromatics, as solvent.

Alkylation of Benzene and Isolation of 1,3,5-Tri-t-butylbenzene

A solution of pure benzene (36 ml., 0.41 mole) in *t*-butyl chloride (189 ml., 1.7 moles) was cooled to −5° and 59 gm. of aluminum chloride added in small portions over a period of two hours. Aliquots removed after one and three-quarter hours and after all the aluminum chloride was added yielded colorless crystalline material mixed with some liquid on decomposition in ice. On crystallization from ethanol, these aliquots yielded a total of 14 gm. of crystalline material (m.p. 60–65°) consisting of a mixture of *p*-di-*t*-butylbenzene and 1,3,5-tri-*t*-butylbenzene. Careful crystallization from ethanol yielded some of the tri-substituted product, m.p. 73°, which did not depress the melting point of an authentic sample. The remainder of the reaction was decomposed in ice water after a total of six hours' reaction. The hydrocarbon layer was extracted with ether and the ether removed on the steam cone. The viscous residue was placed in a refrigerator overnight and some crystalline material (3 gm.) formed. Crystallization from benzene–ethanol yielded colorless crystals which consisted mainly of the high melting hydrocarbon and which melted at 195–200°.

Alkylation of p-Di-t-butylbenzene

Run I—Preparation of the High Melting Hydrocarbons

p-Di-*t*-butylbenzene (47 gm., 0.25 mole) was dissolved in 300 ml. of *t*-butyl chloride and the solution cooled to −5°, causing much of the hydrocarbon to precipitate. Powdered aluminum chloride (66 gm., 0.5 mole) was added in small portions over a two-hour period to this mixture with vigorous stirring. After about five minutes the hydrocarbon redissolved and there was copious evolution of hydrogen chloride. At the end of six hours' reaction in the cold, the evolution of hydrogen chloride subsided and two layers appeared—a yellow liquid layer on top and a thick dark amorphous mass at the bottom of the flask. The reaction mixture was decomposed in crushed ice and the hydrocarbon layer distilled with steam until oily droplets ceased coming over into the distillate. The solid residue was separated from adhering polymeric liquid products on the suction pump by washing with ethanol to yield 20 gm. of colorless crystalline material, m.p. 205–210°. In later runs it was found more convenient to extract the decomposed reaction product directly with ether and proceed with the isolation by concentration of the ether until the hydro-

carbon crystallized. Repeated crystallizations from 1:1 benzene–ethanol yielded colorless needles, m.p. 218.5–219°.

Anal. Found: C, 88.51, 88.63; H, 11.54, 11.45.
C, 88.45, 88.41; H, 11.28, 11.34.
C, 88.47, 88.58; H, 11.35, 11.31.
Molecular weight, 311.6, 309.5, 309.3.
Calc. for $C_{22}H_{34}$: C, 88.51; H, 11.49. Molecular weight 298.5.

Run II—Improved Synthesis of 1,3,5-Tri-t-butylbenzene

Seventy-five grams of *p*-di-*t*-butylbenzene was dissolved in 350 ml. of *t*-butyl chloride and the solution cooled to −3°, causing much of the hydrocarbon to precipitate. Seven grams of aluminum chloride was added during the first half hour to the stirred mixture, the temperature being maintained below 0°. Twenty grams of aluminum chloride was then added in 10 min. causing the semisolid reaction mixture to become liquid and the temperature to lower to −7°. After an additional 10 min., the reaction mixture was carefully decomposed in cold water. The organic layer was extracted with ether, which was removed with excess *t*-butyl chloride on the steam cone. The residue solidified on cooling with an ice–salt mixture to yield 70 gm. (75% yield) of colorless solid. After five recrystallizations from ethanol, this material melted at 74.8–75°. The ultraviolet absorption spectrum (Fig. 2) of this product was practically identical to that of authentic 1,3,5-tri-*t*-butylbenzene prepared in small yield in this laboratory by the condensation of pinacolone.

Anal. Found: Molecular weight 247.7, 245.5, 248.8.
Calc. for tri-butylbenzene 246.4.

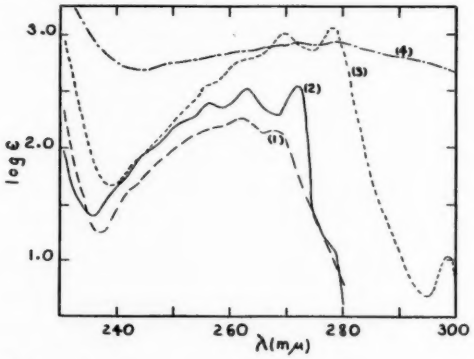

FIG. 2. Ultraviolet spectra.
(1) 1,3,5-Tri-*t*-butylbenzene (m.p. 74.8–75°).
(2) *p*-Di-*t*-butylbenzene (78.5–79°).
(3) The high melting hydrocarbon (m.p. 218.5–219°).
(4) Nitro derivative of the high melting hydrocarbon (m.p. 266.5–267°).

Run III—Intermediates in the Preparation of the High Melting Hydrocarbon

Essentially the same procedure with the same amounts of reagents as in Run II was used except that additional aluminum chloride (7.5 gm.) was added and the reaction was continued for six hours. Aliquots of the reaction

mixture were removed when the mixture became fluid (after 30 min.) and after 45 min. These were decomposed in water and recrystallized from ethanol. The first aliquot contained a mixture of *p*-di-*t*-butylbenzene and 1,3,5-tri-*t*-butylbenzene. The second aliquot (after 45 min.) contained 1,3,5-tri-*t*-butylbenzene, which after one crystallization melted at 73°. At the end of six hours, the remaining reaction mixture was decomposed, extracted with ether, and the excess *t*-butyl chloride removed with the ether on the steam cone. On standing, the liquid residue deposited some crystalline material which yielded 6 gm. of colorless crystals after crystallization from benzene–ethanol, m.p. 211–212°.

Nitration of the High Melting Hydrocarbon

The hydrocarbon (12.6 gm., m.p. 216–218°) was dissolved in a mixture of glacial acetic acid (580 ml.) and acetic anhydride (345 ml.) at 65°. Fuming nitric acid (5 ml., sp. gr. 1.5) was added drop by drop and the reaction mixture was heated at 90° for three hours. On cooling, the product crystallized as pale yellow crystals, 7.3 gm., m.p. 261–262°. Repeated crystallization from benzene–ethanol changed the melting point to 266.5–267°.

Anal. Found: C, 77.75, 77.52; H, 9.57, 9.49; N, 4.04.
Molecular weight, 335.9, 333.9, 334.5.
Calc. for $C_{22}H_{33}NO_2$: C, 76.91; H, 9.68; N, 4.08.
Molecular weight, 343.5.

Preparation and Attempted Hydrolysis of an Amino Derivative

A solution of 5 ml. of concentrated hydrochloric acid in 5 ml. of ethanol was added to a mixture of 0.5 gm. of the nitro derivative with 1 gm. of granulated tin. This mixture was heated on the steam cone under reflux and enough ethanol, saturated with dry hydrogen chloride, was added to give a clear solution. This solution was refluxed for five hours and then allowed to stand for a day. At the end of this time, no crystallization had taken place so that the product was worked up in the usual way by precipitation in water, addition of alkali to dissolve the tin, and extraction with ether. On evaporation of the ether colorless crystals formed, m.p. 247–248°. Crystallization from benzene–ethanol yielded colorless crystals, m.p. 249.2–249.7°.

Anal. Found: C, 85.00, 85.09; H, 11.27, 11.11; N, 4.01.
Calc. for $C_{22}H_{35}N$: C, 84.28; H, 11.26; N, 4.47.

This amine appeared to be almost completely insoluble in concentrated hydrochloric acid but the ultraviolet spectrum, with general absorption maxima at 292($E^{1\%}$ 71.3), 248($E^{1\%}$ 205), and 214 mμ ($E^{1\%}$ 1460), is typical of aromatic amines.

Two grams of this amine was dissolved in 180 ml. of a 1: 5 sulphuric acid – acetic acid solution. The solution was cooled in an ice bath and a solution of 22 gm. of sodium nitrite in 90 ml. of water was added. The reaction mixture was then warmed on the steam cone until evolution of nitrogen ceased, cooled, and extracted with ether. After washing with 10% sodium bicarbonate solution, the excess ether was removed and pale orange crystals formed, m.p. 208–209°. Unsuccessful attempts were made to remove the color from this

product by chromatography on alumina and crystallization with charcoal. The ultraviolet absorption, λ_{max} 265 mμ, $E^{1\%}$ 377, of this compound is much more intense than expected for a phenol and a more detailed study is required before a structure can be suggested for this product.

The Action of Oxidizing Agents on the High Melting Hydrocarbon

Tertiary-butyl groups are known to oxidize with difficulty; for example, Boedtker (2) obtained mainly di-*t*-butylquinone on attempted oxidative degradation of *p*-di-*t*-butylbenzene. Using increased pressure and temperature, Legge (8) succeeded in obtaining terephthalic acid by nitric acid oxidation of *p*-di-*t*-butylbenzene.

Attempts to oxidize the high melting hydrocarbon to a known benzenecarboxylic acid have not been successful. Oxidation with nitric acid in pressure tubes at elevated temperatures yielded acidic material but yields were very small and nitration accompanied oxidation. The use of such oxidizing agents as chromic acid or potassium permanganate in solvents such as acetone, acetic acid, or pyridine has not produced any identifiable benzenecarboxylic acid. It was found, however, that *p*-di-*t*-butylbenzene could be oxidized to terephthalic acid in small yield by heating a powdered mixture of the hydrocarbon with an oxidizing agent such as potassium permanganate in a nitrogen atmosphere at 220°. This procedure has yielded some polycarboxylic acid from the high melting hydrocarbon and, although the degradation was not complete in this case, seems the most promising approach.

ACKNOWLEDGMENTS

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THE SYNTHESIS OF C^{14}-CARBOXYL-LABELLED ω-THIOETHYL FATTY ACIDS[1]

By Anne E. Almond, Anna Mary Burditt, D. E. Douglas,[2] and Jean Eccles

ABSTRACT

Syntheses on a semimicro scale of thioethyl acetic, ω-thioethyl propionic, and ω-thioethyl butyric acids labelled with carbon-14 in the carboxyl group are described. The compounds were chromatographed on filter paper with butanol–water–ammonia as the developing solvent, and the R_F values determined.

In the course of an investigation, at this Institute, of ω-thioalkyl fatty acids as metabolic inhibitors in isolated tissues, carbon-14-carboxyl-labelled thioethyl acetic, ω-thioethyl propionic, and ω-thioethyl butyric acids were required. Experimental details for the synthesis of these labelled molecules on a semimicro scale are described in this communication.

Although ω-thioalkyl acetic acids have been prepared by alkylation of sodium thioglycollate with the appropriate alkyl halide (6) and these and other ω-thioalkyl fatty acids from the sodium salt of a thiol and a ω-halo fatty acid (3, 7) this latter method was only employed for the preparation of thioethyl acetic acid-1-C^{14}.

A more convenient synthetic method for higher homologues involves cyanidation of an ethyl ω-haloethyl sulphide followed by hydrolysis of the resulting nitrile to the acid. This method is not satisfactory when ethyl chloromethyl sulphide is a reactant, owing to the instability of this molecule (2). Ethyl γ-bromopropyl sulphide forms a Grignard reagent, which may be carbonated to yield ω-thioethyl butyric acid. Ethyl β-chloroethyl sulphide, however, failed to form a Grignard reagent.

A method for the paper chromatography of the acids described has been worked out, to assess the radiopurity of the labelled compounds.*

EXPERIMENTAL

Thioethyl Acetic Acid-1-C^{14}

Acetic acid was liberated from 4.8 mM. of sodium acetate-1-C^{14} (199,700 counts total activity) with hydrogen chloride gas according to a previously described high-vacuum procedure (4), and condensed into a reaction flask fitted with a micro dropping funnel and cold-finger condenser. The acetic acid was brominated in the presence of acetyl chloride (0.1 ml.), red phosphorus (20 mgm.), and a crystal of iodine with bromine (0.5 ml.). The latter was added dropwise, while the mixture was heated under reflux (4).

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Contribution from the Division of Atomic Chemistry, Montreal General Hospital Research Institute, Montreal, P.Q.
[2]*In charge, Division of Atomic Chemistry.*
**Recently, a communication (J. Am. Chem. Soc. 76: 3860. 1954) from another laboratory has appeared regarding the antituberculosis activity of some ethylmercapto compounds, including several thioalkyl-fatty acids. No details are given regarding synthetic methods, and presumably carbon-14-labelled compounds were not employed in this investigation.*

After two hours the reaction mixture was hydrolyzed with 2 to 3 ml. of water, and the unreacted bromine removed in a stream of nitrogen. The last traces of bromine were removed with 1 *N* sodium bisulphite. The resulting solution was neutralized to pH 8 with dilute sodium hydroxide, and evaporated *in vacuo*. The residue was dissolved in the minimum amount of water, and 0.62 *M* sodium ethanethiolate (10 ml.) in ethanol was added. The mixture was shaken several times and allowed to stand for 24 hr. and then heated under reflux for three hours, and finally concentrated to dryness *in vacuo*.

The residue was dissolved in water and the aqueous solution extracted continuously with ether for four to five hours. The aqueous layer was acidified to pH 2 with dilute sulphuric acid and extracted continuously overnight with ether. The ethereal solution was dried over sodium sulphate, and evaporated in a stream of nitrogen. The oily residue was distilled from a Späth bulb (bath temperature 100°C.) on the high-vacuum system yielding 0.1555 gm. (21%) of a colorless oil. Specific activity 200 counts per min. per mgm. with 50% counter geometry. $\eta_D^{22°} = 1.4800$.

ω-Thioethyl Propionic Acid-1-C^{14}

Barium carbonate-C^{14} (1 mc. per mM.) was converted to sodium cyanide-C^{14} by Adamson's method (1). To 0.4 mM. of this radiocyanide were added 5 mM. (0.245 gm.) of carrier sodium cyanide, 8 ml. of 80% ethanol, and 5.4 mM. (0.65 ml.) of ethyl β-chloroethyl sulphide. The mixture was heated under reflux for four hours and the solvent was removed *in vacuo*. The remaining oil was hydrolyzed with a solution of 6 *N* hydrochloric acid (5 ml.) and glacial acetic acid (2.5 ml.) in a sealed tube at 100° for 12 hr. The acetic and hydrochloric acids were removed *in vacuo*, and the product dissolved in a few milliliters of water, and extracted exhaustively with chloroform. The chloroform solution was shaken several times with an excess of dilute sodium hydroxide, then with water. The combined alkaline extracts and washings were acidified to pH 2 and extracted four times with chloroform. The combined chloroform solutions were dried over sodium sulphate and filtered. The filtrate was concentrated and the remaining oil was distilled under high vacuum from a Späth bulb. Yield 0.2612 gm. (36%, based on ethyl β-chloroethyl sulphide). Colorless oil. Activity yield: 15.9% from barium carbonate-C^{14}. Specific activity 87,400 c.p.m./mgm. with 50% counter geometry. Calc. for $C_5H_{10}O_2S$: C, 44.76; S, 23.90. Found: C, 44.5; S, 23.8. $\eta_D^{21.5} = 1.4805$.

ω-Thioethyl Propionamide

A sample of non-radioactive ω-thioethyl propionic acid was treated with a slight excess of thionyl chloride, and the mixture heated at 80°C. for 10 min. to complete the reaction. Excess concentrated ammonium hydroxide was added carefully to the resulting acid chloride. The reaction mixture was evaporated to dryness *in vacuo*, and the amide dissolved in isopropyl ether, and recrystallized from the same solvent. Colorless plates, m.p. 64–65°C. Calc. for $C_5H_{11}NOS$: C, 45.08; N, 10.52; S, 24.07. Found: C, 44.3; N, 10.7; S, 24.5.

ω-Thioethyl Butyric Acid-1-C^{14}

(*a*) *Cyanidation of Ethyl γ-Chloropropyl Sulphide and Hydrolysis of the Nitrile*

A mixture of sodium cyanide (0.3 gm.), water (0.5 ml.), ethanol (5 ml.), and ethyl γ-chloropropyl sulphide (0.693 gm.) (5) was heated under reflux for six hours. The ethanol was evaporated *in vacuo*, the residue shaken with water to dissolve salts and then extracted with ether. The ethereal solution was dried with sodium sulphate and evaporated in a bomb tube under a stream of nitrogen. The oily residue was heated with a mixture of 1.5 ml. each of glacial acetic acid and concentrated hydrochloric acid for about three hours at 110°C. The acetic and hydrochloric acids were removed *in vacuo*, and the crude acid purified by the method described above for the preparation of ethyl thioacetic acid. After high-vacuum distillation, the product weighed 0.090 gm. Yield 12%, based on ethyl γ-chloropropyl sulphide. $\eta_D^{22°} = 1.4805$.

(*b*) *Carbonation of the Ethyl γ-Bromopropyl Sulphide Grignard Reagent*

Although ethyl γ-chloropropyl sulphide formed a Grignard reagent only in the presence of ethyl bromide added as promoter, the corresponding bromo-compound was active alone.

Ethyl γ-bromopropyl sulphide was prepared from ethyl γ-hydroxypropyl sulphide and phosphorus tribromide in carbon tetrachloride.

The Grignard reagent was prepared from ethyl γ-bromopropyl sulphide (2.2 gm.), magnesium (0.3 gm.), and dry ether (30 ml.). Carbonation was carried out according to the usual high-vacuum technique with carbon dioxide liberated from 0.9048 gm. (4.6 mM.) of barium carbonate-C^{14} (5.08×10^5 counts total activity) by means of concentrated sulphuric acid. The mixture was allowed to warm slowly to room temperature with occasional shaking after the carbonation at approximately −20°C was assumed to be complete. The magnesio complex was carefully decomposed with excess of dilute sulphuric acid, and the ether layer was removed. The aqueous phase was extracted four times with ether and the combined ethereal solutions were washed with three portions of 0.5 *N* sodium hydroxide (total 15 ml.) then four times with water. The combined alkaline solution and washings were saturated with sodium sulphate, acidified to pH 2, and extracted with ether.

The ethereal solution was dried over sodium sulphate, and the ether removed leaving an oily residue. This residue was distilled in a Späth bulb under high vacuum at a bath temperature of 100° yielding 0.362 gm. of a colorless oil. Specific activity 570 c.p.m./mgm. Yield 53%, based on barium carbonate. Calc. for $C_6H_{12}O_2S$: C, 48.65; S, 21.60. Found: C, 48.7; S, 21.8. $\eta_D^{22.0°} = 1.4805$; mixed R.I. with the product prepared by the previous method $\eta_D^{22°} = 1.4805$. The identity of the compounds obtained by the two procedures was further established by chromatography on filter paper.

A crystalline amide could not be obtained from this compound with thionyl chloride and ammonia.

Paper Chromatography of the Thioethyl Fatty Acids

Approximately 0.1 mgm. quantities of the labelled acids in excess ammonium

hydroxide were chromatographed on Whatman No. 1 filter paper with butanol–water–ammonia by the ascending technique. Butanol (100 ml.) was shaken with water (25 ml.) and ammonium hydroxide (2 ml.) below room temperature, and the butanol layer separated. An open beaker containing concentrated ammonium hydroxide was placed under the battery jar containing the chromatogram. After approximately 15 hr., the chromatograms were removed and dried.

The paper was sprayed with 0.1% aqueous chloramine-T, dried, and sprayed immediately with starch – potassium iodide solution (0.1 gm. soluble starch and 0.1 gm. potassium iodide per 100 ml.). White spots on a blue background indicated the presence of the thio-acids. Radioautographs were obtained by placing the dried chromatograms before treatment with chloramine-T in contact with Eastman No-Screen X-ray film for approximately two weeks.

The following relative R_F values were observed for the regions of maximum concentration:

R_F $C_2H_5SCH_2COOH = 0.34$ $\qquad$ $\dfrac{R\ C_2H_5S\ (CH_2)_2COOH}{R\ C_2H_5\ SCH_2COOH} = 1.30$

$$\frac{R\ C_2H_5S(CH_2)_3COOH}{R\ C_2H_5SCH_2COOH} = 1.55$$

A trace of radioactive contaminant with an R_F of 0.12 was present in the ω-thioethyl propionic and ω-thioethyl butyric acids.

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OXIDE COMPLEXES FORMED IN THE SYSTEMS PLATINUM METALS : ALKALI CARBONATES : OXYGEN[1]

By J. J. Scheer, A. E. van Arkel, and R. D. Heyding[2]

ABSTRACT

On heating mixtures of a platinum metal with an alkali carbonate in different ratios and at different temperatures in an oxygen stream, two kinds of compounds are formed:

(1) the cubic compounds $Na_2Pt_3O_4$ and $Na_2Pd_3O_4$,

and

(2) a series of oxide complexes with compositions ABO_2 or A_2BO_3, both isomorphous with Na_2SnO_3.

Although all metals of the platinum group form oxides, anhydrous complexes of their oxides have not been described. The observation that at high temperatures platinum crucibles are readily attacked by lithium oxide in the presence of air (1) was the first indication that such complexes might be prepared. On investigating the reactions of lithium oxide – platinum black mixtures in oxygen at 1000°C., Bright reported the formation of a product with the approximate formula Li_2PtO_3 (3). Further study of the X-ray pattern has shown that this product was actually a mixture of two or more compounds.

We have found that lithium oxide and platinum react in oxygen even at 400°C. to form single compounds, and that the same products are obtained from lithium carbonate – platinum mixtures. Because of the ease with which carbonate–platinum mixtures could be handled in comparison to those of the oxide (2), all subsequent reactions were restricted to the carbonate. Like the oxide, the carbonate attacks most crucible materials, and it was necessary to use crucibles cut from fused polycrystalline blocks of magnesium oxide.

By replacing the platinum black in the reaction mixtures by other finely divided platinum metals, a number of lithium complexes with Pd, Rh, and Ir were prepared, and by the substitution of Na_2CO_3 for Li_2CO_3, analogous sodium complexes have been obtained.

EXPERIMENTAL

The platinum metals were used in the form of platinum black, prepared by the procedure described in Gmelin (5) (i.e., reduction with sodium formate); palladium black (5); rhodium and iridium sponge, both 99.95% pure, from British Drug Houses Ltd.

Analytically pure sodium carbonate and lithium carbonate (May and Baker) were preheated in oxygen at 400°C. for about five hours.

Portions of metal and carbonate were weighed into a magnesium oxide boat and mixed intimately. This mixture was heated under a slow stream of oxygen which had been dried over sulphuric acid, silica gel, sodium hydroxide, and,

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finally, phosphorous pentoxide. Heating was continued until no further change in weight could be observed.

Debye–Scherrer diagrams of the products were taken in a 9 cm. Unicam camera with Cu K_α radiation.

RESULTS AND DISCUSSION

In the systems Na–Pt–O and Na–Pd–O, the first compounds formed were the cubic phases $Na_xPt_3O_4$ and $Na_xPd_3O_4$ (a_0 = 5.67 Å and 5.64 Å, respectively), the first of which has been described by Waser and McClanahan (8). Experimental and calculated values of $\sin^2\theta$ and of line intensities for one of these structures are given in Table I.

TABLE I

DEBYE–SCHERRER DIAGRAM OF $Na_xPd_3O_4$ (CUBIC)
vs, very strong; s, strong; m, moderate; w, weak; vw, very weak

hkl	$\sin^2\theta$ (calc.)	$\sin^2\theta$ (obs.)	*I* (calc.)	*I* (obs.)
110	0.0371	0.0387	16.0	m–s
200	0.0742	0.0769	15.5	m–s
210	0.0928	0.0944	100.0	vs
211	0.111	0.113	31.9	s
220	0.149	0.151	0.3	w
300, 221	0.167	0.169	0.2	vw
310	0.186	0.188	7.6	m
222	0.223	0.225	35.5	s
320	0.241	0.244	39.1	s
321	0.260	0.262	25.3	s
400	0.297	0.299	27.0	s
411, 330	0.334	0.336	6.3	m
420	0.371	0.374	3.9	m
421	0.390	0.393	50.1	vs
510, 431	0.4826	0.4850	10.8	m
520, 432	0.5383	0.5407	67.0	vs
521	0.5568	0.5583	16.0	m
440	0.5939	0.5951	39.0	s
530, 433	0.6311	0.6327	7.8	m
600, 442	0.6682	0.6678	5.0	m
610	0.6867	0.6875	26.1	m–s
611, 532	0.7053	0.7054	28.2	m–s

On heating mixtures of the molecular proportions metal : carbonate ::1:1 for about 20 hr. at 600°–1000°C., the A_2BO_3 compounds Li_2RhO_3, Na_2IrO_3, Na_2PtO_3, and Li_2PtO_3 were obtained. These compounds are isomorphous with a series of complexes of quadrivalent elements, Li_2TiO_3, Na_2TiO_3, Na_2SnO_3, and Na_2PbO_3, investigated by Lang (6). Typical X-ray data are given in Table II.

It was not possible to prepare Na_2PdO_3 by this method, although quadrivalent palladium appears to exist in the phase Li_2PdO_3.

TABLE II

DEBYE-SCHERRER DIAGRAM OF Na_2PtO_3 (ORTHORHOMBIC)

hk l	$\sin^2\theta$ (calc.)	$\sin^2\theta$ (obs.)	*I* (calc.)	*I* (obs.)
00 6	0.0212	0.0209	58.3	s
02 0	0.0286	0.0283	3.5	w
0012	0.0850	0.0845	81.7	s
20 $\bar{2}$	0.0837			
20 $\bar{4}$	0.0907	0.0922	56.8	s
20 $\bar{8}$	0.119	0.120	100.0	s
2010	0.140	0.141	38.3	m
00$\bar{1}$8	0.191	0.193	16.0	w
20$\bar{1}$4	0.197	0.197	15.1	w
2016	0.232	0.233	46.7	s
06 0	0.244	0.244	73.6	s
06 $\bar{6}$	0.2653	0.2663	65.9	s
20$\bar{2}$0	0.3173	0.3176	23.4	w–m
40 2	0.3277	0.3287	76.9	s
06$\bar{1}$2				
40 $\bar{4}$	0.3347	0.3341	9.6	w
0024	0.3398	0.3400	18.9	w
40 $\bar{8}$	0.3631	0.3624	25.5	m
20$\bar{2}$2	0.3669			
40$\bar{1}$0	0.3843	0.3845	6.7	w

The rhodium mixtures first formed the compounds $LiRhO_2$ and $NaRhO_2$, and it seems probable that the latter compound is also transformed into the A_2BO_3 compound Na_2RhO_3, but its crystal structure has not yet been established.

It has been found that both ABO_2 compounds are isomorphous with the A_2BO_3 compounds (Table III). The lattice of the compound $LiRhO_2$ consists of alternating layers of lithium and rhodium atoms separated by oxygen layers. From this structure the crystal of the A_2BO_3 type is formed by the

TABLE III

DEBYE-SCHERRER DIAGRAM OF $NaRhO_2$

hk l	$\sin^2\theta$ (calc.)	$\sin^2\theta$ (obs.)	*I* (calc.)	*I* (obs.)
00 6	0.0220	0.0232	34.1	s
20 $\bar{2}$	0.0854	0.0865	35.8	s
0012	0.0880	0.0896	27.6	m–s
20 $\bar{4}$	0.0928	0.0947	49.6	s
20 $\bar{8}$	0.122	0.123	100.0	s
2010	0.144	0.145	29.1	m–s
20$\bar{1}$4	0.2028	0.204	21.7	m–s
00$\bar{1}$8	0.199			
2016	0.240	0.242	44.8	s
06 0	0.250	0.250	74.2	s
06 $\bar{6}$	0.2723	0.2728	29.5	m–s
20$\bar{2}$0	0.3275	0.3292	21.2	m
40 2	0.3344	0.3375	65.7	s
06$\bar{1}$2	0.3383			
0024	0.3520	0.3481	14.1	w
40 $\bar{8}$	0.3711	0.3705	15.1	m
20$\bar{2}$2	0.3788	0.3817	6.8	w–m
40$\bar{1}$0	0.3931	0.3924	5.5	w
06$\bar{1}$8	0.4483	0.4490	42.8	m–s
40$\bar{1}$4	0.4518			
40$\bar{1}$6	0.4885	0.4884	13.0	w–m
20$\bar{2}$6	0.4962	0.4978	13.7	w–m

substitution of one lithium and two quadrivalent rhodium ions for three trivalent rhodium ions in the lattice, leading to the formula $Li_{4/3}\,Rh_{2/3}\,O_2 = 2/3\,Li_2RhO_3$. A very similar substitution has been observed in fluorides of the $NaLaF_4$ type, which may be written as $Na_{3/2}\,La_{3/2}\,F_6$ or $(Na_{3/2}\,La_{1/2})LaF_6$, and are isomorphous with Na_2ThF_6 (9).

According to Lang (6), the structure of Na_2SnO_3 can be described by reference to a monoclinic cell, as well as to a larger orthorhombic cell, three times the volume of the former. In Table IV the parameters of both cells are given for the isomorphous ABO_2 and A_2BO_3 complexes; whether the symmetry is orthorhombic or pseudorhombic will be left undecided.

TABLE IV

LATTICE CONSTANTS OF THE ABO_2 AND A_2BO_3 COMPOUNDS EXPRESSED IN ANGSTROM UNITS
rh = orthorhomic; mo = monoclinic; $h\,k\,(3l+h)_{rh} = h\,k\,l_{mo}$

	Na_2SnO_3*	Na_2PtO_3	Na_2IrO_3	$NaRhO_2$	Li_2SnO_3*	Li_2PtO_3	Li_2RhO_3	$LiRhO_2$
a	5.50	5.39	5.39	5.34	5.29	5.17	5.12	5.23
b	9.53	9.34	9.33	9.22	9.19	8.96	8.87	9.07
c_{rh}	32.51	31.67	31.59	31.11	29.61	28.86	28.73	28.52
c_{mo}	10.99	10.71	10.68	10.32	10.03	9.77	9.73	9.66
c/a	5.93	5.88	5.86	5.83	5.59	5.58	5.61	5.45
β	99°6′	99°40′	99°41′	99°44′	100°1′	100°10′	100°6′	100°22′

*Cf. Lang (6).

The structure of these compounds is closely related to that of the hexagonal complexes of one of the iron metals, i.e. $LiNiO_2$ (high temperature modification) and $NaNiO_2$ (4), the difference lying in the relative positions of the metal layers. Both groups can be derived from a cubic close packed arrangement of oxygen ions, with the metal ions in the octahedral interstices. By a slightly different arrangement of the metal atoms the cubic structure of $LiFeO_2$ is obtained.

In all of these groups the metal ions are surrounded by six oxygen ions, and in this respect are completely different from the compounds of the $Na_xPt_3O_4$ type, in which all platinum atoms are in the center of square PtO_4 groups. There can be no doubt that the Pt—O bonds are due to dsp^2 hybridization, as in PtO (7); however there are two kinds of platinum ions in the lattice according to the formula $Na_xPt^{2+}_{1+x}Pt^{3+}_{2-x}O_4$. The range of the parameter x has not yet been determined.

We intend to continue this investigation by including the remaining metals of the iron and platinum groups as well as other alkali metals.

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THE PURIFICATION OF SEDOHEPTULOSE[1]

By L. Ujejski[2] and E. R. Waygood[3]

ABSTRACT

Methods involving paper and cellulose column chromatography are described for the purification of sedoheptulose phosphate from the mother liquor obtained from *Sedum spectabile* by the procedure of LaForge and Hudson. The beginnings of crystallization with subrectangular arrangement of rod-like longulites of free sedoheptulose have been obtained by chromatographic separation in phenol from an equilibrium mixture of sedoheptulosan and sedoheptulose prepared by treating the former with 3 *N* hydrochloric acid at room temperature. A spraying reagent for carbohydrates containing ketose units is described.

The 7-carbon sugar sedoheptulose has been known since 1917 when it was isolated by LaForge and Hudson (7) from *Sedum spectabile* in the form of a syrup. Ettel (4) in 1932 established its configuration as D-altroheptulose. The free ketose has not yet been obtained in crystalline form, but Pratt *et al.* (9) in 1952 established the formula of the crystalline non-reducing anhydride, sedoheptulosan, as 2,7-anhydro-β-D-altroheptulopyranose.

The intermediate role of the sugar and its phosphorylated derivative in the metabolic processes of plants and animals has been recently investigated in many laboratories (5, 11). The subject matter of the present communication is, however, concerned solely with the purification of the free ketose and its phosphorylated derivative.

The previous assumption that sedoheptulosan and sedoheptulose may be determined by the same color reactions (10) has been proved correct and this also applies to sedoheptulose phosphate.

Sedoheptulose phosphate has been isolated from the 'mother liquor' obtained from leaves of *S. spectabile* by the method of LaForge and Hudson (7). The yellow-green dense liquid was chromatographed on filter paper and most of the yellow impurities remained at the starting line. Sedoheptulose phosphate was detected by orcinol reagent (1) and corresponding spots were eluted and determined spectrophotometrically by the cysteine–sulphuric and the carbazole – sulphuric acid reactions (3). The former reagent gave a transient yellow color which changed to orange after 48 hr., maximum density being at 510 mμ, characteristic of sedoheptulosan (10). Carbazole–sulphuric produced the expected red color, but the mixture did not show the constant ratio D490/D400 = 1.90 characteristic of the anhydride, indicating the presence of impurities. A second chromatographic separation, using 100 spots each containing 200 μgm. initially, produced a glassy yellow-product after elution and evaporation *in vacuo*. Larger amounts were purified on cellulose column

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and this procedure also produced a glassy product that, after drying, could be ground to a yellowish white powder, which became sticky on exposure to air. Determination of bound phosphate indicated that this substance was sedoheptulose monophosphate.

Free sedoheptulose was not isolated directly from the mother liquor; however this sugar was separated chromatographically from the equilibrium mixture produced by incubating sedoheptulosan with 3 *N* hydrochloric acid for 12 hr. at room temperature. Difficulties were encountered since no spraying reagent could be found for sedoheptulosan. Of numerous solvents tried, phenol was found to be preferable for the separation of sedoheptulose from the equilibrium mixture. Chromatographic development in this manner produced two spots with the orcinol reagent. Similar spots were obtained by developing a solution of the equilibrium mixture in ethanol – butanol – *N*/20 hydrochloric acid (110: 10: 20).

It is known that the behavior of phenol as a solvent (6) in paper chromatography differs from other organic solvents giving greater R_f values than could be expected from the water content. "The explanation probably lies in the fact that phenols can form compounds with sugars analogous to these formed by water, so that the distribution of a sugar between the aqueous phenolic solvent and water–cellulose complex will depend not only on the water present in each phase but also on the phenol." (6).

When sedoheptulose phosphate and sedoheptulosan were developed in phenol each gave one spot in different positions with the orcinol reagent, whereas the equilibrium mixture gave two spots in corresponding positions (Fig. 1). The explanation for this lies in the fact that performed sedoheptulose

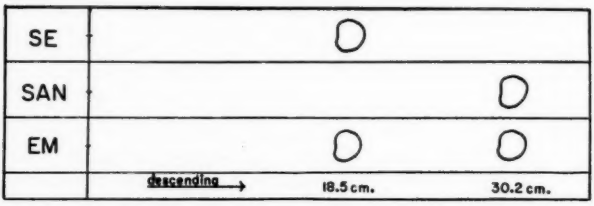

FIG. 1. Paper chromatogram showing the movement of sedoheptulose (SE), sedoheptulosan (SAN), and the equilibrium mixture (EM) in phenol.

in the equilibrium mixture moves slower in this solvent than sedoheptulosan, but the anhydride forms a new equilibrium mixture in its position on the paper in the presence of phenol. The newly formed free sugar is detected by the orcinol reagent but it does not separate from the anhydride during development. Therefore the two spots correspond to sedoheptulose and the newly formed equilibrium mixture of sedoheptulosan and sedoheptulose. This property of separation of sedoheptulose from the equilibrium mixture has served as a basis for its isolation in pure form.

Spots corresponding to free sedoheptulose were cut out, eluted with water, passed through Amberlite anion exchange resin, and evaporated to dryness *in vacuo*. A crystalline substance was obtained which was identified as sedo-

heptulose by its ósazone. Complete crystallization of a large quantity of sedoheptulose was not obtained, but only the beginnings of crystallization of sedoheptulose (Fig. 2). The experiments indicated that in order to obtain larger amounts of the substance it would be advantageous to combine both cellulose column and ion exchange chromatography.

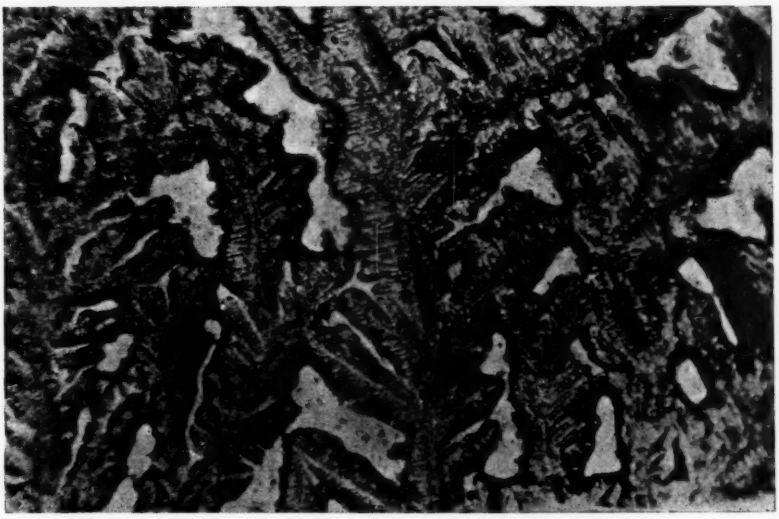

FIG. 2. The subrectangular arrangement of rod-like longulites of free sedoheptulose crystals.

Dische (2) prepared the equilibrium mixture by treating sedoheptulosan with 1% hydrochloric acid on a steam bath for 30 min. and obtained three different spots with the orcinol reagent on his chromatograms in phenol. Noggle (8) used 0.2 *N* hydrochloric acid on a steam bath for 30 min. and detected four spots under the same conditions. He suggested that the first two R_f 0.45 and 0.70 represented sedoheptulose and sedoheptulosan and the other two spots unknown carbohydrates.

It is possible that the heat treatment may produce other anhydrides of sedoheptulose (9). This is supported by Noggle's (8) use of Dowex 50 in the hydrogen form to prepare the equilibrium mixture in place of hot 0.2 *N* hydrochloric acid. Under these conditons he detected only sedoheptulose and sedoheptulosan in the equilibrium mixture. Our separations were concerned only with mixtures produced at room temperature.

In the course of studying various spraying reagents for sedoheptulosan, Dische's reagent (3) was modified and adapted for use on paper. Cysteine and carbazole dissolved in butanol and trichloracetic acid gave a brownish spot with sedoheptulose. This spray had a wide application and gave distinctly colored spots with reducing and non-reducing mono- and poly-saccharides containing ketose units. It was found also that phloroglucinol gave a bluish green spot with sedoheptulose.

EXPERIMENTAL

Purification of Sedoheptulose Phosphate by Chromatography

The purification of sedoheptulose syrup was accomplished either by paper or cellulose column chromatography.

In the former procedure, 20 mgm. of the syrup was dissolved in 1 ml. of water and aliquots of 10 μl. were placed on Whatman No. 1 filter paper and developed for 36 hr. in butanol–ethanol–water (10: 1: 2). The resulting spots, located by orcinol, were cut out and eluted with 400 ml. water. This solution was evaporated *in vacuo*, redissolved in 1 ml. water, and chromatographed again in butanol – acetic acid – water (4: 1: 2). Near to the end of the evaporation a yellow-white precipitate appeared which formed a glassy product when dry. Recovery was 80%. The filter papers were previously washed with 0.03 *N* hydrochloric acid, then with water to neutrality, and afterwards with 0.5 *N* sodium hydroxide followed by water to neutrality.

In the latter procedure, 20 ml. of a yellow solution of 1% sedoheptulose sirup in methanol was passed through a column (25 cm. × 1.5 cm.) of Whatman No. 1 cellulose. The yellow ring that remained on the column was eluted with water until the orcinol reaction became negative. The solution was evaporated *in vacuo* and a glassy yellow-white powder was obtained.The product was readily pulverized in a mortar but eventually became sticky during exposure to air.

In solution, free phosphate was formed after hydrolysis with *N* hydrochloric acid for three hours on a steam bath. The phosphate produced corresponded to a monophosphoric ester of sedoheptulose. Further purification of this material by paper chromatography did not alter its physical properties.

The first methyl alcoholic effluent was colorless and gave no orcinol reaction. After evaporation to dryness a small amount (1%) of amorphous white product was obtained. The substance was sparingly soluble in water, m.p. 138°C., and gave no reaction for sugars. The nature of this product is as yet unknown.

Separation of Crystalline Sedoheptulose from the Equilibrium Mixture

Crystalline sedoheptulosan was used as the starting material using descending paper chromatography with a 90% solution of freshly distilled phenol as the solvent. Sixty milligrams of sedoheptulosan were treated with 0.5 ml. of 3 *N* hydrochloric acid at room temperature for 12 hr., 3.5 ml. water were added; thus 1 ml. contained 15 mgm. of the equilibrium mixture (sedoheptulose and sedoheptulosan). Aliquots of 10 μl. of this solution were developed for 30 hr. on paper. The components of the equilibrium mixture were identified by running parallel spots of sedoheptulose phosphate and sedoheptulosan. Two spots were observed on the path of the equilibrium mixture, one corresponding to sedoheptulose and the other to sedoheptulosan. Apparently sedoheptulose phosphate and sedoheptulose move at the same rate. The identification of sedoheptulosan by the orcinol spray is due to the formation of a new equilibrium mixture in this spot.

Elutions of spots from 3 ml. of a solution containing 45 mgm. of the equilibrium mixture in hydrochloric acid were combined and passed through

Amberlite IR4B(OH) and evaporated *in vacuo*. Fig. 2 shows the beginnings of crystallization and the subrectangular arrangement of rod-like longulites of free sedoheptulose obtained by this separation. Seven milligrams of the free ketose was obtained corresponding to approximately 20% of sedoheptulose in the equilibrium mixture assuming 80% recovery.

Aliquots of 10 μl. of sedoheptulose phosphate as well as the equilibrium mixture were also developed in butanol – ethanol – $N/20$ hydrochloric acid (100: 10: 20). As with phenol one spot was obtained with sedoheptulose phosphate and two with the equilibrium mixture using the orcinol reagent.

New Spraying Reagent for Carbohydrates Containing Ketose Units

Dische's reagent, a mixture of cysteine, carbazole, and sulphuric acid, was modified as follows:

Cysteine hydrochloride, 1.5 gm., was dissolved in 6 ml. of water, 15 gm. of trichloracetic acid were added, made up to 100 ml. with butanol, and 0.12 gm. of carbazole was added. The sprayed papers were heated for 10 min. at 85°.

The following carbohydrates containing ketose units show brown spots: dihydroxyacetone, fructose, sorbose, hexosediphosphate, ascorbic acid, sedoheptulose, sucrose, turanose, melizitose, stachyose (two spots), and inulin.

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THE REACTION OF ACTIVE NITROGEN WITH HYDRAZINE[1]

By G. R. Freeman and C. A. Winkler

ABSTRACT

Hydrazine was completely destroyed by active nitrogen, at both 150°C. and 480°C., up to a hydrazine flow rate of about 22×10^{-6} mole per sec., whereas ammonia production was small at hydrazine flow rates below about 12×10^{-6} mole per sec. Thus it appears that ammonia is formed in secondary reactions only. The results indicate that NH_2 radicals rather than hydrogen atoms may be prominent in secondary reactions. Comparison of the rate of hydrazine destruction with the rate of production of hydrogen cyanide from ethylene indicates that excited nitrogen molecules do not make a large contribution to the chemical reactivity of active nitrogen.

INTRODUCTION

The reaction of active nitrogen with ammonia (5) gave strong indication that more than one chemically reactive species exists in active nitrogen.* It was therefore of interest to study the analogous reaction with the related compound, hydrazine.

EXPERIMENTAL

The apparatus used was essentially the same as that described in earlier papers (3, 6) with one modification. Since hydrazine is a liquid (b.p. 113°C.) with a vapor pressure of 10 mm. at 20°C., it was not convenient to store it as a gas. It was stored as a liquid in a cylindrical bulb (cross sectional area = 28 sq. cm.) which was connected to the flowmeter through a 7 liter ballast volume. The storage bulb was immersed in a thermostat regulated at 20.95 ± 0.05°C. The rate of flow of hydrazine into the reaction vessel was varied by placing jets of different sizes in the flowmeter.

The molecular nitrogen flow rate was 9.2×10^{-5} mole/sec., corresponding to a pressure of 1.5 mm. Hg in the reaction vessel. In some experiments, where hydrogen atoms replaced active nitrogen, the molecular hydrogen flow rate was 8.0×10^{-5} mole per sec., which gave an operating pressure of 1.0 mm. Hg.

Anhydrous hydrazine (99%) was very kindly contributed by the Mathieson Chemical Corp.

The only products from the reactions of hydrazine with either active nitrogen or hydrogen atoms are nitrogen, hydrogen, and ammonia. The condensable products were collected in a trap containing standard sulphuric acid, immersed in liquid nitrogen. After the solution had been melted, the total amount of base in the products was determined by titration to methyl red end point. The hydrazine content of the solution was then determined by

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Quebec, with financial assistance from the National Research Council of Canada.

**It seems probable that one active species is nitrogen atoms, while some other component such as N_2^* or N_3 might also contribute to the activity.*

titration with iodate (1). The difference between the amounts of total base and hydrazine gave the amount of ammonia.

Some comparative experiments were also made with the ethylene – active nitrogen system. In these, hydrogen cyanide was analyzed by the Liebig–Dénigès method (7).

RESULTS AND DISCUSSION

The yellow afterglow of active nitrogen was observable in the tube leading from the reaction vessel to the cold trap at all hydrazine flow rates until that corresponding to the maximum rate of decomposition of hydrazine was approached (up to about 20×10^{-6} mole per sec.). In the ethylene – active nitrogen reaction, the yellow afterglow was gradually replaced by the cyanogen flame; at 420°C., no nitrogen afterglow could be observed at flow rates of ethylene above about 1×10^{-6} mole per sec. However, at this temperature the cyanogen flame was observable in the tube leading from the reaction vessel to the cold trap at all ethylene flow rates until that corresponding to the maximum rate of production of hydrogen cyanide was approached (up to about 13×10^{-6} mole per sec.). Thus the presence, in the tube leading to the cold trap, of the nitrogen afterglow in the hydrazine reaction, and the cyanogen flame in the ethylene reaction, seem to indicate the persistence of active nitrogen in the two reactions. Since active nitrogen reacts with ammonia to only about 15% the extent to which it reacts with ethylene (5) or hydrazine, the persistence of the yellow afterglow in the ammonia reaction up to flow rates much higher than that corresponding to the maximum rate of destruction

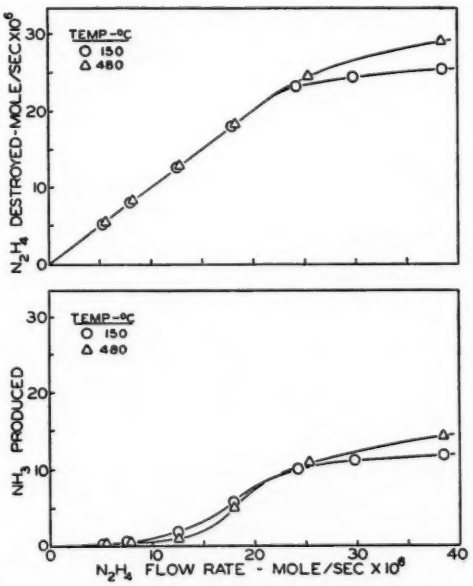

FIG. 1

of ammonia may be taken as further indication of the presence of two active species in active nitrogen.*

The relations between the hydrazine flow rate, the amount of hydrazine destroyed, and the corresponding amount of ammonia produced are shown in Fig. 1 for different temperatures. At both 150°C. and 480°C. hydrazine was completely destroyed up to a flow rate of about 22×10^{-6} mole per sec. The maximum rate of destruction increased by about 14% when the temperature was increased from 150°C. to 480°C.

From the shape of the ammonia production curves, it would appear that ammonia is formed only by reaction of hydrazine with a product of the hydrazine – active nitrogen reaction. The amount of ammonia produced at 480°C. was smaller than the amount produced at 150°C. in the range of lower flow rates, but the reverse was true at the higher flow rates (above about 20×10^{-6} mole per sec.). This was probably due to a greater increase in the rate of the active nitrogen – hydrazine reaction than in the rates of the secondary reactions. Thus there would be less hydrazine available for secondary reactions until all the active nitrogen was consumed. The possibility that ammonia was produced in the initial reaction, and destroyed by subsequent reaction with excess active nitrogen is ruled out because the maximum rate of destruction of ammonia by active nitrogen in the absence of any other reactant would be about 3×10^{-6} mole per sec. under similar conditions (5). It is

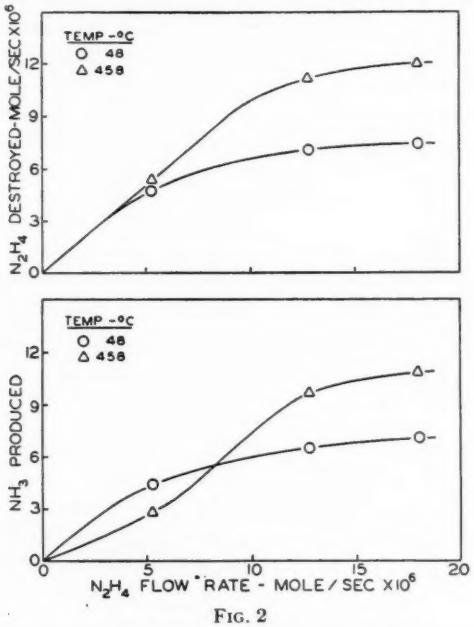

FIG. 2

**This persistence of the yellow afterglow implies the persistence of a species of nitrogen which contains at least 170 kcal. of energy, i.e. a species with sufficient energy to generate $N_2B^3\Pi_g$ molecules.*

probable, also, that the component in active nitrogen capable of reacting with ammonia would be rapidly removed by reaction with hydrazine.

There was a possibility that hydrogen atoms might be formed from the attack of active nitrogen on hydrazine. Although the hydrogen atom – hydrazine reaction has been studied (2, 4), some experiments were made in the present apparatus to permit a more direct estimate of the extent to which hydrogen atom reactions might contribute to the over-all active nitrogen – hydrazine reaction.

The hydrazine – hydrogen atom reaction was studied at 48°C. and 458°C., and the results are shown in Fig. 2. At 48°C., one mole of ammonia was produced for each mole of hydrazine destroyed. At 458°C., $(1-\alpha)$ mole of ammonia was formed for each mole of hydrazine destroyed, where $0 < \alpha < 1$. The value of α decreased, i.e. ammonia production increased, as the hydrazine flow rate was increased, probably in parallel with a decrease in hydrogen atom concentration available for secondary reactions as the hydrazine flow rate was increased (due to direct reaction between H and N_2H_4). This would imply that the decrease in yield of ammonia with increase of temperature was due to attack of hydrogen atoms on ammonia, or on an intermediate from which ammonia might be derived. The results can be explained by the following reactions:

		ΔH(kcal.)*	
$H+N_2H_4$	$\rightarrow H_2+N_2H_2$	−(8 to 13)	[1*a*]
	$\rightarrow NH_3+NH_2$	−39	[1*b*]
$2N_2H_3$	$\rightarrow N_2+2NH_3$	−(144 to 154)	[2]
$H+NH_3$	$\rightarrow H_2+NH_2$	+4	[3]
$NH_2+N_2H_3$	$\rightarrow N_2+H_2+NH_3$	−(117 to 122)	[4]
$2NH_2$	$\rightarrow N_2+2H_2$	−90	[5*a*]
$2NH_2+M$	$\rightarrow N_2H_4+M$	−68	[5*b*]
$2H+M$	$\rightarrow H_2+M$	−102.7	[6]
$H+N_2H_3+M$	$\rightarrow N_2H_4+M$	−(90 to 95)	[7*a*]
$H+N_2H_3$	$\rightarrow N_2+2H_2$	−(113 to 118)	[7*b*]

**To calculate heats of reactions, the following dissociation energies and heats of formation were used:*

D_{N_2} = 9.765 ev. = 225.0 kcal. (Hendrie, J. M. J. Chem. Phys. 22: 1503. 1954).
D_{H_2} = 4.455 ev. = 102.7 kcal. (Beutler, H. Z. physik. Chem. B29: 315. 1935).
D_{H-NH_2} = 4.65 ev. = 107 kcal. (Devins, J. C. and Burton, M. J. Am. Chem. Soc. 76: 2618. 1954).
$\Delta H_f(NH_3)$ = −10.94 kcal. (Handbook of chemistry and physics).
$\Delta H_f(N_2H_4)$ = 22.7 kcal. (Reference (1), this paper).
From these values, the following were calculated:
Average N—H bond energy in NH_3 = 92.5 kcal.
Average N—H bond energy in NH_2 = 85.3 kcal.
Total bond energy in N_2H_4 = 407.7 kcal.
$D_{H_2N-NH_2}$ = 67.7 kcal.
Total bond energy in NH_3 = 277.5 kcal.
If it is then assumed that the average $H_2N—NH_2$ bond energy = 60 kcal., the average $H—N_2H_3$ bond energy = 87 kcal. Therefore, it was assumed that $D_{H-N_2H_3}$ = 90 to 95 kcal. It was also assumed that D_{H-NH} = 90 kcal., and D_{H-N} = 81 kcal. (these values were approximated from the total bond energy in NH_3 and D_{H-NH_2}).

If reaction [1*b*] were the predominant initial reaction, one ammonia molecule

would be formed from each hydrazine molecule destroyed. This would prohibit the reaction

$$NH_2+N_2H_4 \rightarrow NH_3+N_2H_3 \qquad \Delta H = -(12 \text{ to } 17)\text{kcal.} \quad [8]$$

since ammonia production never exceeded hydrazine consumption on a mole basis. At 458°C. and low hydrazine flow rates a large proportion of the ammonia formed by reaction [1*b*] would have to be subsequently destroyed by hydrogen atoms (see Fig. 2). This would seem to require that the ammonia so formed be excited, since the rate of destruction previously observed with hydrogen atoms at 440°C. (5) was much too small to explain the large value of α found at 458°C. and low hydrazine flow rates.

Although reaction [1*a*] is less favorable energetically than reaction [1*b*], its occurrence might be expected by analogy with the corresponding hydrogen atom reaction with ethane,*

$$C_2H_6+H \rightarrow C_2H_5+H_2 \qquad \Delta H = -4.7 \text{ kcal.} \quad [9a]$$

which is generally accepted over the more energetically favorable reaction

$$C_2H_6+H \rightarrow CH_4+CH_3 \qquad \Delta H = -18 \text{ kcal.} \quad [9b]$$

If reaction [1*a*] were the predominant initial reaction, the high value of α at 458°C. and low hydrazine flow rates could be explained readily by the combined effect of reactions [3] and [7*b*]. It is also possible that some N_2H_3 radicals are destroyed on the walls at high temperatures to yield nitrogen and hydrogen but no ammonia. Although the NH_2 radicals might have a significant concentration at high temperatures and low hydrazine flow rates, the hydrazine concentration should be very low because of reaction [1*a*]; hence reaction [8] following upon [1*a*] should not occur to any appreciable extent.

There are two possible exothermic reactions between nitrogen atoms and hydrazine,

$$N+N_2H_4 \rightarrow N_2+H_2+NH_2 \qquad \Delta H = -91 \text{ kcal.} \quad [10a]$$
$$\rightarrow N_2+H_2+NH+H \qquad \Delta H \approx -1 \text{ kcal.} \quad [10b]$$

If N_3 were an active species, it would react in the same manner as N, but the corresponding reactions would be less exothermic by the amount of the N_2—N bond energy in N_3. Since hydrazine reacts rapidly with active nitrogen the most probable mechanism for the reaction, if N and N_3 are assumed to be the reactive species, would seem to be:

		ΔH(kcal.)	
$N+N_2H_4$	$\rightarrow N_2+H_2+NH_2$	−91	[10*a*]
$NH_2+N_2H_4$	$\rightarrow NH_3+N_2H_3$	−(12 to 17)	[8]
$2N_2H_3$	$\rightarrow N_2+2NH_3$	−(144 to 154)	[2]
$NH_2+N_2H_3$	$\rightarrow N_2+H_2+NH_3$	−(117 to 122)	[4]
$2NH_2$	$\rightarrow N_2+2H_2$	−90	[5*a*]
$2NH_2+M$	$\rightarrow N_2H_4+M$	−68	[5*b*]

**The bond strengths involved here were taken from a paper by Szwarc, M. Chem. Revs. 47: 75. 1950.*

If N_2^* were a reactive species, it could react with hydrazine without producing ammonia directly, or it might react to produce ammonia in a secondary reaction with the N_2H_3 radical derived from N atom attack on hydrazine:

$$N_2^* + N_2H_4 \rightarrow 2NH_2 + N_2 \quad [11]$$
$$N_2^* + N_2H_3 \rightarrow NH_3 + N_2 + N. \quad [12]$$

Reaction [12] would be followed by [10*a*]. Each N_2^* would result in the decomposition of only one hydrazine molecule.

Since ammonia is a product of secondary reactions only, it is possible to separate the effect of the secondary reactions from that of the total reaction. The ethylene – active nitrogen reaction at 451°C. was used to estimate the total active nitrogen concentration because hydrogen cyanide production from other hydrocarbons studied thus far has never been found to exceed that from ethylene. The maximum amount of hydrogen cyanide produced was 19.0×10^{-6} mole per sec. It may be assumed that if N_3 were a reactive species, each N_3 would yield one molecule of hydrogen cyanide, and if N_2^* were a reactive species, each N_2^* would yield two molecules of hydrogen cyanide. Therefore, if N_3 and N were the reactive species, the maximum amount of hydrazine decomposed without the production of ammonia should be approximately equal to the maximum amount of hydrogen cyanide produced from ethylene. If, however, N_2^* makes a predominant contribution to the activity of active nitrogen, the maximum amount of hydrazine decomposed without the production of ammonia should be much smaller than the maximum amount of hydrogen cyanide produced from ethylene.

The only product of the active nitrogen hydrazine reactions considered above which could react further with hydrazine is the NH_2 radical. Therefore, each hydrazine molecule decomposed in a secondary reaction (reaction [8], followed by reactions [2] and [4]) would yield two molecules of ammonia. Since, in the hydrogen atom – hydrazine reaction, at low temperature, little, if any, N_2H_3 was destroyed without production of ammonia, it is reasonable to assume that the same is true for the active nitrogen reaction at 150°C. Therefore, by subtracting one half of the amount of ammonia formed at 150°C. from the total amount of hydrazine destroyed at this temperature, for hydrazine flow rates of 24×10^{-6} mole per sec. and higher, it was estimated that 18.8×10^{-6} mole per sec. of hydrazine was destroyed by direct reaction with active nitrogen. This estimate must be increased by an amount corresponding to the amount of hydrazine regenerated by reaction [5*b*]. It was not possible to calculate from the present results the extent to which reaction [5*b*] occurred under the conditions of the reaction reported in this paper. Therefore, it may be concluded only that the maximum rate of destruction of hydrazine by active nitrogen was at least 18.8×10^{-6} mole per sec. The close agreement of this figure with that for hydrogen cyanide production in the ethylene – active nitrogen reaction indicates that N_2^* makes, at best, only a small contribution to the chemical reactivity of active nitrogen, and therefore, that reactions [11] and [12] only occur to a small extent or not at all.

The increase in the maximum amount of hydrazine destroyed when the temperature was increased from 150°C. to 480°C. was presumably due to an increased rate of decomposition of hydrazine in reaction [8].

The long "induction period" in the ammonia production curves (1) indicates that reaction [10*a*] is much faster than reaction [8], which would be expected.

The ammonia production curves tend to level off at a value corresponding to only about 35% of the NH_2 radicals reacting according to reaction [8]. This indicates that the NH_2 radicals are removed quite rapidly by the combined effects of reactions [4], [5*a*], and [5*b*].

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THE REACTION OF METHYL RADICALS WITH ACETALDEHYDE[1]

By R. E. Dodd[2]

ABSTRACT

The rates of formation of methane and ethane in the photodecomposition of acetaldehyde have been measured between 391 and 564°K. The rate constant for the reaction

$$CH_3+CH_3CHO \rightarrow CH_4+CH_3CO$$

was found to be

$$k = 10^{10.8}\, T^{\frac{1}{2}}\, e^{-8000/RT} \text{ moles}^{-1} \text{ cm.}^{+3} \text{ sec}^{-1}.$$

The abstraction of hydrogen from acetaldehyde by methyl radicals, reaction [1], was first proposed by Leermakers (10) as a step in the photolysis of acetaldehyde

$$CH_3+CH_3CHO \rightarrow CH_4+CH_3CO. \qquad [1]$$

Undoubtedly it makes an important contribution to the rate of formation of methane, R_M, in the photolysis, and at high temperatures it predominates. For some time the activation energy, E_1, for reaction [1] was identified with the over-all activation energy, E_p, for the high-temperature photolysis. It is now clear that this involved the assumption of too simple a mechanism for the photolysis for which the reported values (1, 8, 10, 11) of E_p lay in the range 8.3–10.0 kcal./mole.

Under conditions where reaction [1] is the only source of methane $R_M = k_1[CH_3][CH_3CHO]$ but any attempt to state the methyl radical concentration in terms of the light absorption involves specific assumption about the possible reactions of the methyl radicals. For an estimate of k_1 it is safer to derive the methyl radical concentration from the rate of formation of ethane, R_E. This involves the one assumption that any ethane formed arises solely from reaction [2]

$$CH_3+CH_3 \rightarrow C_2H_6. \qquad [2]$$

Then $[CH_3] = k_2^{-\frac{1}{2}} R_E^{\frac{1}{2}}$ and hence

$$k_1k_2^{-\frac{1}{2}} = R_M R_E^{-\frac{1}{2}} [CH_3CHO]^{-1}.$$

Since, however, under the conditions of low light intensity which were used, reaction [1] is so much faster than reaction [2] ethane was not detected in the products of photolysis without the aid of the mass spectrometer. Volman and Brinton (13) were able to attain a higher concentration of methyl radicals by the thermal decomposition of di-*t*-butyl peroxide in the presence of acetaldehyde and were thus able to measure R_E and hence $k_1k_2^{-\frac{1}{2}}$. With $E_2 = 0$ (7) they obtained $E_1 = 7.5$ kcal. per mole over a narrow temperature range

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[2]*National Research Council of Canada Postdoctorate Fellow, 1952–53. Present address: The Chemical Laboratories, King's College, University of Durham, Newcastle-upon-Tyne, England.*

and with a rather large percentage conversion of reactant. Ausloos and Steacie (2) have now extended the temperature range using methyl radicals from the photolysis of azomethane and obtained a value $E_1 = 6.8$ kcal. per mole.

Although ethane was first detected by mass spectrometer in the products of the photolysis of acetaldehyde (3, 4, 6) it was not measured so as to give absolute values of R_E to allow calculation of $k_1k_2^{-\frac{1}{2}}$ (with the possible exception of one point (12) at 300°C.). During the course of work on the photolysis of acetone at low pressures (5) it became evident that the right conditions could be obtained for the formation of ethane from acetaldehyde in amount sufficient for measurement in a gas burette. Thus $k_1k_2^{-\frac{1}{2}}$ could be accurately measured in the direct photolysis. The results of experiments under these conditions are presented in this paper. Although it is somewhat higher than the value of E_1 (6.8 kcal. per mole) obtained from further measurements on the direct photolysis by Ausloos and Steacie, a value ($E_1 = 8.0$ kcal. per mole) lower than the over-all activation energy, E_p, is favored.

EXPERIMENTAL

The apparatus used is described in detail elsewhere (5). The reaction cell was of quartz, 100 cm. in length and 1185 cm.^{3} in effective volume. All experiments were with about 10 mm. of aldehyde. The length of vessel allowed sufficient absorption of light at this pressure of aldehyde, and the volume allowed the accumulation of sufficient products without going beyond about five per cent decomposition.

Two sets of experiments were performed: experiments 57–66 with a Corning 9700 filter, whose transmission exceeds 50% at wave lengths greater than 2900 Å; experiments 79–85 with Corning 9863 and 7740 filters, where the transmission exceeds 50% only above 3100 Å. The reduction in intensity in the second set is thus largely at the expense of higher energy quanta between 2900 and 3100 Å. For each set a separate calibration of the photometer was made using, as standard, the carbon monoxide yield from acetone above 125°C. Carbon monoxide, hydrogen, and methane were separated from ethane by a liquid nitrogen trap. Carbon monoxide and hydrogen were burnt on hot copper oxide. The resultant carbon dioxide was measured after the water was trapped with dry ice. The water, representing hydrogen, was measured by difference.

RESULTS AND DISCUSSION

The results are listed in Table I. The ratio $\rho = R_M R_E^{-\frac{1}{2}}[CH_3CHO]^{-1}$ is shown as a function of temperature in Fig. 1, which also includes the similar measurements reported by Ausloos and Steacie (2) for 35 mm. of aldehyde. It is evident that the three sets approach, with increasing temperature, a line of slope corresponding to about 8 kcal. per mole.

The curvature at low temperatures indicates that other sources of methane are becoming significant by comparison with the reduced rate of reaction [1]. Although more possibilities will have to be considered, it is convenient first to discuss the curvature in terms of a primary intramolecular rearrangement

TABLE I

SUMMARY OF RESULTS

Expt.	°K.	Pressure, mm.	I_a	R_{CO}	R_M	R_{H_2}	R_E	ρ	$k_1k_2^{-\frac{1}{2}}$
Corning 9700; 2900–3300 Å; $k_0' = 0.4$									
59	406	9.18	42.3	37.7	26.3	3.33	9.29	23.8	8.34
60	406	9.22	43.0	37.4	28.3	3.37	9.08	25.9	9.95
57	430	9.67	39.1	52.5	37.0	3.22	10.7	31.3	17.4
58	430	9.69	42.7	50.8	36.9	3.51	10.5	31.6	16.6
61	471	10.64	40.4	92.5	72.0	7.68	13.3	54.4	40.8
62	482	10.85	43.1	112.2	88.2	12.5	14.6	63.8	49.5
63	505	11.2	36.9	146.8	119.2	17.0	16.0	83.4	70.3
64	537	12.0	45.7	263	224	28.0	21.3	135	119
66	562	12.7	37.8	393	340	32.0	24.8	188	172
65	564	12.7	40.4	438	356	—	24.4	198	180
Corning 9860 and 7740; 3130 Å; $k_0' = 0.1$									
79	391	10.00	24.5	7.8	5.8	0.30	1.39	12.1	6.85
72	412	9.83	28.5	10.6	8.4	0.57	1.56	17.6	11.3
74	420	10.35	28.2	12.8	10.1	0.63	1.85	18.9	13.3
76	438	10.05	22.3	16.3	13.5	0.66	1.83	27.0	21.9
78	451	11.15	22.7	22.5	18.9	1.08	2.21	31.9	27.3
81	461	11.21	21.9	28.5	23.9	1.63	2.71	37.1	32.7
84	469	9.44	16.4	26.9	22.7	1.19	2.43	45.0	40.3
85	480	10.40	16.1	36.2	31.1	1.38	2.79	53.4	48.9

Units: aldehyde pressure, mm.; I_a, 10^{-12} Nhv cm.$^{-3}$ sec.$^{-1}$; rates, 10^{-12} moles cm.$^{-3}$ sec.$^{-1}$; and $k_1k_2^{-\frac{1}{2}}$, mole$^{-\frac{1}{2}}$ cm.$^{3/2}$ sec$^{-\frac{1}{2}}$.

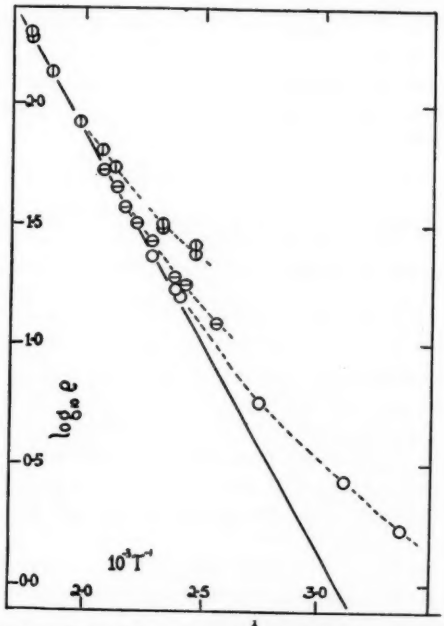

FIG. 1. The temperature dependence of $R_M/R_E^{\frac{1}{2}}[CH_3CHO]$.
⊕ Expts. 57–66; ⊖ Expts. 72–85; ○ Ausloos and Steacie, direct photolysis.

(quantum yield, k_0') so that the relevant steps in the mechanism are:

$$\begin{aligned} &CH_3CHO+h\nu \rightarrow CH_4+CO, && k_0'I_a \\ &CH_3CHO+h\nu \rightarrow CH_3+CHO, && k_0I_a \\ &CH_3+CH_3CHO \rightarrow CH_4+CH_3CO, && k_1 \\ &CH_3+CH_3 \rightarrow C_2H_6. && k_2 \end{aligned}$$

These lead to the equation:

$$\rho = k_1k_2^{-\frac{1}{2}}+k_0'I_aR_E^{-\frac{1}{2}}[CH_3CHO]^{-1}$$

in which, evidently, the last term becomes relatively smaller at high temperatures. The Arrhenius plot for ρ should tend with increasing temperature towards a straight line corresponding to that for $k_1k_2^{-\frac{1}{2}}$, as is the case. Further support is given to this interpretation of the curvature in Fig. 1 by the fact that, so far as my experiments are concerned, the curvature is proportionately less (i.e., commences at lower temperatures) in the set of experiments in which, other things being equal, the light intensity was lower. Furthermore it is possible to estimate the values of the quantum yield, k_0', which will give a straight line Arrhenius plot for $k_1k_2^{-\frac{1}{2}}$. Thus, $k_0' = 0.4$ for the experiments with light of wave length down to 2900 Å and 0.1 for those more closely limited to 3130 Å. These are reasonable values in accordance with the view that k_0' is greater at shorter wave lengths. It is not possible to make the same assessment for the experiments done on the direct photolysis by Ausloos and Steacie (2) since they did not measure I_a: it is probable that the intensities lay between those of the two sets of experiments reported here.

If account is taken of the fact that the formation of ethane is pressure dependent (5, 9) a correction must be applied to k_2 since these experiments were performed at 10 mm. pressure. This amounts to depressing the curves by 0.05 logarithmic units throughout. The two sets of corrected numbers are shown in Fig. 2 where they lie well on the line:

$$\log_{10}(k_1k_2^{-\frac{1}{2}}) = 4.65+\tfrac{1}{4}\log_{10}T-8000/2.3\,RT.$$

With the accepted value for k_2 (at high pressures) (7) this gives:

$$k_1 = 6.3 \times 10^{10}.T^{\frac{1}{2}}.e^{-8000/RT} \text{ moles}^{-1} \text{ cm.}^3 \text{ sec}^{-1}.$$

Included in Fig. 2 are results obtained by Volman and Brinton (13) and by Ausloos and Steacie (2) using methyl radicals derived from the thermal decomposition of di-*t*-butyl peroxide and from the photolysis of azomethane. The agreement in the overlapping temperature range is very good. On the other hand Ausloos and Steacie's figures, taken independently, lie on a line yielding $E_1 = 6.8$ kcal./mole, a difference from 8.0 kcal. which probably lies outside systematic experimental error. But the points at 27 and 51°C. lie above this line and the extra methane has been attributed to wall reactions. It may be suggested that any reaction leading to such amounts of additional methane at these temperatures would still make a significant contribution to the methane formation at higher temperatures and may thus explain the whole of the discrepancy between the two sets of data.

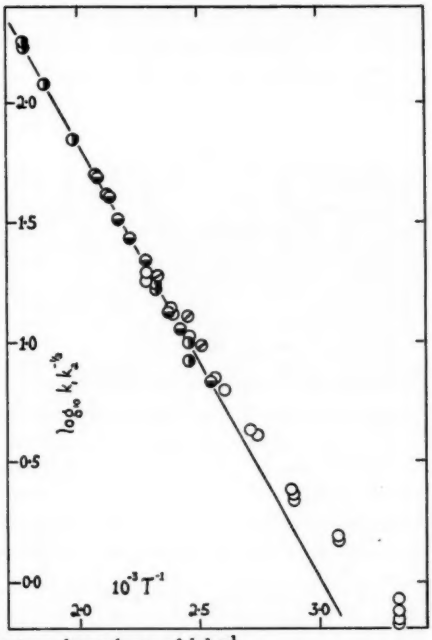

FIG. 2. The temperature dependence of $k_1 k_3^{-\frac{1}{2}}$.
◑ Expts. 57–66; ◒ Expts. 72–85; ○ Ausloos and Steacie, methyls from azomethane; ⊘ Volman and Brinton, methyls from di-*t*-butyl peroxide.

Production of methane at the walls may make some contribution to the curvature in Fig. 1, though this becomes less likely at higher temperatures. The reaction [3]

$$CH_3 + CHO \rightarrow CH_4 + CO \qquad [3]$$

is, however, an important alternative to the primary rearrangement as an explanation of the curvature. The values given to k_0' are thus upper limits. The occurrence of reaction [3] might be tested by high intensity flash experiments with mixtures of $CH_3.CHO$ and $CD_3.CDO$: under conditions where only radical–radical reactions occur the presence of CH_3D and of CD_3H in the products would confirm reaction [3].

The confirmation of a low value of E_1 (and, consequently, of a more reasonable frequency factor, A_1) in experiments on the photolysis of acetaldehyde itself makes it certain that the photolysis mechanism is complex. Since at high temperatures reaction [1] is nevertheless the principal methane-producing reaction the complications probably lie in other reactions which affect the relationship between the methyl radical concentration and the light intensity.

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THE HYDROLYSIS OF PHOSPHATE DIESTERS WITH BARIUM HYDROXIDE[1]

By C. W. Helleiner[2] and G. C. Butler

ABSTRACT

The rate of alkaline hydrolysis of diphenyl phosphate has been found to be increased by the presence of barium. Similarly, desoxyribonucleate (DNA), which is not hydrolyzed rapidly by hot sodium hydroxide, is hydrolyzed by barium hydroxide. Only a very small proportion of the total phosphorus of either diphenyl phosphate or DNA is converted to inorganic phosphate during this hydrolysis. In addition to hydrolysis of the phosphate ester bonds of DNA, hot alkali also causes the deamination of desoxycytidylic acid residues and probably of the amino-purine nucleotides as well.

INTRODUCTION

For the study of nucleotide sequences in desoxyribonucleate (DNA) it seemed desirable to use a nonspecific hydrolytic agent to bring about partial degradation of the polymer to fragments whose identity could be established. Statements by Bredereck and Müller led us to believe that in hot alkaline solution, DNA would be degraded to tetranucleotides. Preliminary experiments showed that such degradation did not occur, but the work of Cherbuliez and Leber (9, 10) suggested that the reaction might proceed more readily if barium hydroxide were used instead of sodium hydroxide. It was found that barium hydroxide did apparently catalyze the hydrolysis of phosphate esters, and a more detailed study of the reaction with a model compound was undertaken.

EXPERIMENTAL

Materials and Methods

DNA was prepared from calf thymus glands by the method of Hammarsten (12).

Diphenyl phosphoric acid was prepared by hydrolysis of diphenyl phosphoryl chloride, which was kindly supplied by Dr. E. Baer. The product was recrystallized by dissolving it in hot water, acidifying the solution, and chilling.

Desoxycytidylic acid was prepared by the method described by Hurst, Marko, and Butler (14).

Desoxyuridylic acid was kindly given to us by Mr. I. G. Walker, who made it by deaminating desoxycytidylic acid with nitrous acid.

Determinations of phenol were done by the method of Folin and Ciocalteu (11) as modified by Kunkel and Tiselius (16).

Determinations of total phosphorus and inorganic phosphate were done by the method of King (15).

Hydrolysis of Diphenyl Phosphate

The hydrolysis of diphenyl phosphate was studied because this compound resembles DNA in containing a doubly-esterified phosphate group. The rate

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Contribution from the Department of Biochemistry, University of Toronto, Toronto, Ontario.
[2]*National Research Council Fellow.*

of appearance of phenol and inorganic phosphate was determined in a solution containing 0.025% diphenyl phosphoric acid, sodium hydroxide, and barium chloride in the concentrations shown in Figs. 1 and 2. For each determination

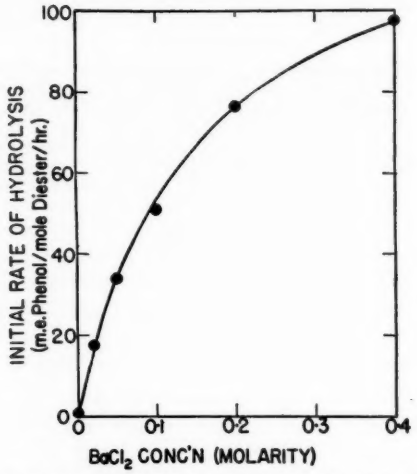

FIG. 1. Initial rate of release of phenol from diphenyl phosphate (0.025% solution) in the presence of 0.1 *N* sodium hydroxide and various concentrations of barium chloride.

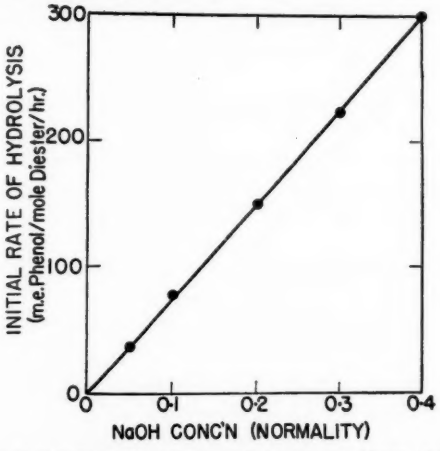

FIG. 2. Initial rate of release of phenol from diphenyl phosphate (0.025% solution) in the presence of 0.2 *M* barium chloride and various concentrations of sodium hydroxide.

a 3 ml. sample was cooled, acidified with 10% hydrochloric acid, and the barium precipitated by adding 0.5 ml. of saturated sodium sulphate solution. The barium sulphate was removed by centrifugation and total phosphorus, inorganic phosphate, and phenol were measured in the supernatant liquid. Most experiments were carried out in a boiling water bath; for the experiment

to investigate the effect of temperature on the reaction, the reaction mixture was sealed into glass tubes, which were heated in an oil bath.

The release of inorganic phosphate was in every case very slow compared to the release of phenol. The inorganic phosphate in various experiments amounted to from 0.1 to 0.4% of the total phosphorus after five hours' heating. Phenol, on the other hand, was released rapidly. Fig. 1 shows the effect of varying the concentration of barium chloride in the reaction mixture on the initial rate of appearance of free phenol. It can be seen that only very slow hydrolysis took place in the absence of barium chloride; as more barium was added to the digest, the rate of hydrolysis became greater. Fig. 2 shows the effect of varying the concentration of sodium hydroxide in the reaction mixture. The initial rate of appearance of free phenol was found to be directly proportional to the concentration of sodium hydroxide for concentrations up to 0.4 *N*. Evidently diphenyl phosphate was hydrolyzed to phenol and monophenyl phosphate, but very little hydrolysis of the resulting monophenyl phosphate occurred.

TABLE I

EXTENT OF HYDROLYSIS OF DIPHENYL PHOSPHORIC ACID (FINAL CONCENTRATION 0.025%) BY VARIOUS HYDROLYTIC AGENTS AFTER FIVE HOURS AT 100°

Hydrolytic agent	Extent of hydrolysis	
	Moles free phenol per mole diester	Moles inorganic phosphate per mole diester
0.01 *N* NaOH, plus:		
0.1 *M* Na_3AlO_3	0.176	0.004
0.43 *M* NH_4OH	0.006	—
approx. 0.01 *M* $Ca(OH)_2$ (saturated)	0.039	—
approx. 0.007 *M* $Mg(OH)_2$ (saturated)	0.012	—
0.1 *M* $BaCl_2$	0.260	0.003
no addition	0.0103	0.008

TABLE II

EXTENT OF HYDROLYSIS OF DIPHENYL PHOSPHORIC ACID (FINAL CONCENTRATION 0.025%) BY 0.1 *N* SODIUM HYDROXIDE, 0.2 *M* BARIUM CHLORIDE AFTER ONE HOUR AT VARIOUS TEMPERATURES

Temperature, (degrees C.)	Moles free phenol (per mole diester) released
90	0.039
100	0.102
110	0.224
120	0.446

Table I shows the effect of other di- and tri-valent cations on the hydrolysis of diphenyl phosphate. None of these was found to be as effective as barium in accelerating the reaction. The effect of temperature on the reaction, shown in Table II, conforms to the relation of Arrhenius.

Hydrolysis of DNA

The progress of the hydrolysis of DNA was followed by measuring the rate of increase in solubility in 10% trichloroacetic acid of the phosphorus-containing compounds of the mixture (17). Solutions containing 5 mgm. per ml. of DNA and the concentrations of sodium hydroxide and barium hydroxide shown in Table III were heated on a boiling water bath. Samples were treated as described for diphenyl phosphate to remove the barium, with precautions to avoid precipitation of DNA. To the solution from which the barium had been removed was added an equal volume of ice-cold 20% trichloroacetic acid. After filtration, the phosphorus contents of the original solution and of the filtrate were determined. This gave a measure of the solubility of the alkali-treated material in 10% trichloroacetic acid. Inorganic phosphate was also determined on the filtrate.

Table III shows that the hydrolysis of DNA apparently proceeds in accordance with expectations based on the findings with diphenyl phosphate. DNA is degraded to acid-soluble fragments with the production of only a little inorganic phosphate, and the reaction is accelerated by increasing the concentration of either barium or hydroxide. It was also found that the reaction could be speeded up by conducting it at 110°C. in a sealed reaction vessel.

TABLE III

EXTENT OF HYDROLYSIS OF DNA BY VARIOUS CONCENTRATIONS OF SODIUM AND BARIUM HYDROXIDE AFTER TWO HOURS AT 100°

NaOH conc'n, *N*	$Ba(OH)_2$ conc'n, *N*	% acid-soluble	% inorganic phosphate
Constant hydroxide concentration			
0.30	0.30	43	1.0
0.50	0.10	43	1.1
0.55	0.05	35	1.8
0.58	0.02	26	1.3
0.60	0.00	7.5	2.4
Constant barium chloride concentration (0.05 M)			
1.0		58	3.3
0.5		30	1.4
0.2		10	1.5
0.1		9	0.6

Deamination of Desoxycytidylic Acid

It seemed probable that the drastic conditions employed for hydrolyzing DNA would not only cleave phosphodiester linkages but would also deaminate the purine and pyrimidine bases. This possibility was investigated by subjecting desoxycytidylic acid to the same conditions as those required to hydrolyze

DNA, and measuring the rate of deamination of this compound to desoxyuridylic acid.

Shugar and Fox (18) showed that uracil has an isosbestic point at 235 mμ, i.e., changes in pH of solutions of uracil from values of about 1 to 7 do not affect the extinction at this wavelength. Cytosine, on the other hand, exhibits a large change in the extinction at 235 mμ when the pH of the solution is changed from 1 to 7. Therefore any difference in the extinction at 235 mμ between pH 1 and 7 of a solution containing only desoxycytidylic and desoxyuridylic acids should be proportional to the quantity of desoxycytidylic acid present, and independent of that of desoxyuridylic acid. This relationship was verified experimentally by adjusting the pH of solutions containing varying proportions of desoxycytidylic and desoxyuridylic acids to 1.0 and 7.0 using a Beckman model G pH meter. The optical densities of the solutions were read at 235 mμ on a Beckman model DU spectrophotometer. It was found that

$$58.8\ \Delta\epsilon = \mu\text{gm. of desoxycytidylic acid per ml.}$$

where $\Delta\epsilon$ is the difference in optical density of two solutions containing the same quantity of desoxycytidylic and desoxyuridylic acids, adjusted to pH 1 and 7 respectively. $\Delta\epsilon$ is independent of the concentration of desoxyuridylic acid.

The extent of deamination of desoxycytidylic acid (final concentration 0.05%) under various conditions, as measured by this method, is shown in Table IV. It is evident that under no conditions could DNA be hydrolyzed by

TABLE IV

DESTRUCTION OF DESOXYCYTIDYLIC ACID (FINAL CONCENTRATION 0.05%) UNDER VARIOUS CONDITIONS

Time of digestion	Temp. (°C.)	Hydrolytic agent 0.05 *M* $BaCl_2$ plus:	NH_4OH conc'n, *N*	% destruction of desoxycytidylic acid
5 hr.	91	0.2 *N* NaOH	—	59
5 hr.	91	0.1 *N* NaOH	—	34
90 min.	110	0.1 *N* NaOH	—	77
45 min.	110	0.1 *N* NaOH	—	32
60 min.	110	0.1 *N* NaOH	0.4	67

alkali without considerable deamination of desoxycytidylic acid, and probably of the other nucleotides as well. This finding is in keeping with that of Hurst (13), who found that all the nucleotides are readily deaminated in hot alkaline solution. It was thought that speeding up the hydrolytic reaction by raising the temperature, or adding a large amount of ammonium hydroxide to the reaction mixture might reduce the amount of deamination, but neither of these measures brought about any appreciable reduction.

DISCUSSION

The catalysis of hydrolysis of phosphate esters, as well as anhydrides of phosphoric acid by di- and tri-valent cations in alkaline solution, has been reported by several groups of investigators. Bamann *et al.* (1–7) have noted this type of effect with hydroxides of lanthanum and other rare earths; their

results are difficult to assess, however, since the insolubility of these hydroxides makes it impossible to state their concentration in the system. These workers have interpreted their findings as "phosphatase models". They have compared the specificities and pH optima of various rare earth hydroxides in hydrolyzing a large number of esters and anhydrides of phosphoric acid.

Cherbuliez and Leber (10) studied the effect of calcium hydroxide on the hydrolysis of esters of phosphoric acid, though without quantitative analysis of the products of the reaction. They observed a catalytic effect with calcium, but not with barium hydroxide.

The statement of Bredereck and Müller (8) concerning the lability of DNA to sodium hydroxide has been shown to be misleading. Weygand, Wacker, and Dellweg (19) have reported the hydrolysis of DNA by lead hydroxide; these workers isolated desoxyribosides from the hydrolyzate in yields ranging from 1–6%.

Although we have demonstrated that increasing the concentration of barium hydroxide leads to more rapid hydrolysis of phosphate esters, we have not found any explanation for the phenomenon. We see no virtue in making analogies with the equally poorly understood phenomenon of enzymic catalysis. Cherbuliez and Leber (10) propose a mechanism which involves the formation of phosphonium ions, but if it were valid, monovalent cations should also catalyze the hydrolysis of phosphate esters, and we have demonstrated that they do not.

The fact that an increase of sodium hydroxide concentration in the absence of barium does not appear to lead to any acceleration of the hydrolysis, while an increase of either barium or sodium hydroxides when both are present does lead to such an acceleration, suggests that the true catalytic agent may be undissociated barium hydroxide. In order to be certain of this it would be necessary to have measurements of the degree of dissociation of barium hydroxide at high temperatures and hydroxyl ion concentrations.

ACKNOWLEDGMENT

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SYNTHESIS AND CHARACTERIZATION OF D-XYLOFURANOSE-5-PHOSPHATE[1]

By J. L. Barnwell, W. A. Saunders, and R. W. Watson

ABSTRACT

Phosphorylation of 1,2-*O*-isopropylidene-D-xylose with diphenylphosphorochloridate yielded crystalline 1,2-*O*-isopropylidene-D-xylofuranose-5-diphenylphosphate. Subsequent hydrogenolysis in glacial acetic acid over Adams' catalyst quantitatively removed phenyl groups as shown by infrared analysis. Mild hydrolysis in acetic acid for two hours at 80°C. removed the isopropylidene grouping, and D-xylose-5-phosphate was isolated as an amorphous barium salt. A yield of 81% of theoretical was obtained from 1,2-*O*-isopropylidene-D-xylose, or an over-all yield of 72% from xylose. The product was characterized through its amorphous barium, disodium, and dipotassium salts, and its crystalline dibrucine and distrychnine salts.

One of the earliest observations on phosphoryl group migration in sugar phosphates was that of Levene and Raymond (10). In attempts to prepare xylose-3-phosphoric acid (8, 9, 10, 11) as a basis for testing Robinson's suggestion that xylose was the pentose component of nucleic acid (13), 5-acetyl-, 5-benzoyl-, and 5-benzyloxycarbonyl-1,2-*O*-isopropylidene-D-xylose were separately phosphorylated with phosphorus oxychloride (10, 11). After removal of the acyl and isopropylidene groups by hydrolysis, the only product obtained was D-xylose-5-phosphate. Migration of the phosphoryl group from carbon-3 to carbon-5 at some stage of the synthesis was explained by postulating the formation of a cyclic intermediate (11). The possible significance of this exchange reaction in biological systems, as well as fundamental interest in the mechanism, have indicated the need for more complete investigation. In the present communication a revised synthesis for the preparation of D-xylose-5-phosphate is reported.

The only previously reported synthesis of D-xylose-5-phosphate was that of Levene and Raymond (10, 11). Phosphorylation of 1,2-*O*-isopropylidene-D-xylose (20 gm.) with phosphorus oxychloride in dry pyridine at −30°C., followed by drastic hydrolysis in 2 *N* sulphuric acid at 80°C. for two hours yielded 5.5 gm. of barium D-xylose-5-phosphate. This represented 14.3% of the theoretical yield from 1,2-*O*-isopropylidene-D-xylose, or approximately 12% from xylose. In the present study substitution of diphenylphosphorochloridate as the phosphorylating agent in dry 2,6-lutidine followed by hydrogenolysis and mild hydrolysis in acetic acid has increased the yield from 1,2-*O*-isopropylidene-D-xylose to 81%. The over-all yield of barium-D-xylose-5-phosphate from xylose in a typical synthesis was 72%.

The phosphorylation product, 1,2-*O*-isopropylidene-D-xylose-5-diphenylphosphate, may be obtained pure by recrystallization from carbon tetrachloride. Its elementary analysis conforms to the theoretical, and it has an extremely sharp melting point (within 0.2°). Infrared analysis of finely ground crystals

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in a nujol mull shows characteristic phenyl absorption bands and a strong hydroxyl peak. The sharp melting point suggests the absence of isomeric phosphates and the greater reactivity of the primary hydroxyl indicates preferential phosphorylation in the 5-position. The assumption that phenyl groups would prevent double phosphorylation through hindrance is borne out by the elementary and infrared analyses.

If the phosphorylated product is homogeneous, acid conditions during hydrogenolysis and hydrolysis would favor retention of the phosphoryl group in position 5. The final product in the form of its barium salt has no inorganic or acid-labile phosphate, and it consumes 3.0 moles of oxidant when reacted with sodium periodate at pH 4.7. Barium, phosphorus, and pentose are close to theoretical for a monophosphorylated compound. However, when subjected to periodate oxidation either in 0.05 *N* sulphuric acid or at pH 4.7, samples prepared by different methods release from 3 to 7% of formaldehyde. The amount of formaldehyde released may be correlated with the pH during acetic acid hydrolysis. No exact estimate of the purity of the product may be made at present, although the samples used for characterization probably contain 97% D-xylose-5-phosphate.

Crystalline derivatives of D-xylose-5-phosphate have not previously been reported. Levene and Raymond (10) performed an elementary analysis on the barium salt and determined specific rotations for the barium and disodium salts. In the present study attempts to prepare crystalline metal salts were unsuccessful. Dilithium, disodium, dipotassium, as well as the barium salts precipitated from water and ethanol in amorphous form. In order to place identification on a crystalline basis the dibrucine and distrychnine salts were prepared and their constants determined.

EXPERIMENTAL

Preparation of 1,2-O-Isopropylidene-D-xylose and Diphenylphosphorochloridate

Pure 1,2;3,5-di-*O*-isopropylidene-D-xylose (115 gm.) was prepared from pure xylose in 87% yield (6). Hydrolysis of this product gave 1,2-*O*-isopropylidene-D-xylose in 99% yield (12). By molecular distillation this compound was obtained as colorless crystals with $[\alpha]_D^{26} = -19.3$ ($c = 5.70$ in water) (14). Diphenylphosphorochloridate in essentially pure form (212 gm.) was prepared from 1.1 moles of freshly distilled phosphorus oxychloride and 2 moles of redistilled phenol (79% yield) (3, 4).

Phosphorylation of 1,2-O-Isopropylidene-D-xylose

Crystalline 1,2-*O*-isopropylidene-D-xylose (5.25 gm.) was mixed with reagent benzene and the benzene distilled to remove traces of water. After removal of the benzene freshly distilled anhydrous 2,6-lutidine (60 gm.) (2) was added, the mixture shaken until solution was complete, and cooled to −20°C. with dry ice. Dry diphenylphosphorochloridate (15 gm.) was added dropwise over a period of 10 min. from a separatory funnel fitted with a drying tube. After standing for two days at 5°C. the reaction mixture was filtered, and the crystalline 2,6-lutidine–hydrochloride washed several times with cold 2,6-lutidine. After evaporation of the filtrate to a thick sirup water was added

and the distillation to dryness repeated to remove 2,6-lutidine. The resulting sirupy residue was dissolved in 100 ml. of chloroform, washed nine times with 100-ml. portions of water, and the washings discarded. The chloroform solution was evaporated to a sirup *in vacuo* at 35°C., the last traces of chloroform being removed by azeotropic distillation after addition of ethanol. Colorless needles were obtained by crystallization from ether. (Yield 10.8 gm., 93% of theoretical.) After it was recrystallized from carbon tetrachloride, washed with petroleum ether, and dried *in vacuo* at 50°C., the pure 1,2-*O*-isopropylidene-D-xylose-5-diphenylphosphate had m.p. 102.2°–102.4°: $[\alpha]_D^{20} = +10.8$ ($c = 2.21$ in chloroform). (Found: C, 56.10; H, 5.38. Calc. for $C_{20}H_{23}O_8P$: C, 56.84; H, 5.49.) The compound was soluble in chloroform and acetone, slightly soluble in ether, ethanol, and carbon tetrachloride, and insoluble in water and petroleum ether.

*Hydrogenolysis and Hydrolysis of 1,2-O-Isopropylidene-*D*-xylose-5-diphenylphosphate*

Low pressure hydrogenation of the crystalline phosphorylation product (2.0 gm.) over 0.20 gm. of Adams' catalyst (1) in 60 ml. of glacial acetic acid was complete in 115 min. The solution was filtered and the filtrate, after adjustment to pH 1.5 ± 0.2 with water, was hydrolyzed at 80 ± 2°C. for two hours. Acetic acid was removed by vacuum distillation, the sirup dissolved in 90 ml. of water, and 0.3 *N* barium hydroxide added until the pH reached 8.5. Removal of inorganic phosphate was effected by centrifuging the slightly turbid aqueous solution before the addition of four volumes of ethanol. After standing at 5°C. for two hours the colorless barium salts were separated by centrifugation and washed with 75% ethanol and ether before drying *in vacuo* over anhydrone. (Yield, 1.33 gm.) Concentration of the supernatant liquid to 20 ml. on a Craig evaporator at 30°C., readjustment of the pH to 8.5 with saturated aqueous barium hydroxide, and reprecipitation with four volumes of ethanol recovered 0.245 gm. for a total yield of 1.575 gm. (90% of theoretical).

Characterization of D*-Xylose-5-phosphate*

Data obtained by analysis of the barium salt isolated from the reaction mixture agreed with theoretical values calculated for a monophosphorylated pentose. (Found: Ba, 37.51; P, 8.54; pentose 40.2. Calc. for $C_5H_9O_8PBa$: Ba, 37.59; P, 8.58; pentose 41.0.) Pentose was estimated by the modified method of Cohn and Volkin (5). A series of analyses at different time intervals showed maximum color development with D-xylose-5-phosphate in 15 min. so that the heating period was reduced from 20 min. to 15 min.

Free xylose-5-phosphoric acid had $[\alpha]_D^{20} = +25.0$ ($c = 2.00$ in water) and the specific rotation remained unchanged for two days. The disodium salt, prepared by addition of a stoichiometric quantity of dilute sodium carbonate solution to the barium salt, had an initial $[\alpha]_D^{20}$ of $+10.0$ ($c = 2.00$ in water, pH 7.2) which changed with time in a levorotatory direction to $+7.0°$, $+4.4°$, $+1.9°$, $0.0°$, and $-1.0°$ after one two, three, four, and five days respectively. The dipotassium salt had $[\alpha]_D^{20} = +13.4$ ($c = 2.09$ in water, pH 8.9). In

half-saturated sodium tetraborate solution the specific rotation of the disodium salt increased gradually in a dextrorotatory direction. After two, three, four, and five days the initial rotation of $[\alpha]_D^{20} = +5.5$ ($c = 2.00$ in water) had changed to +6.8°, +8.5°, +10.1°, and +10.6° respectively. Several attempts were made to prepare crystalline metal salts of xylose-5-phosphoric acid. Under the conditions explored, the dilithium, disodium, dipotassium, and barium salts were always obtained in amorphous form from either water or ethanol.

Acid-labile phosphate, measurable by King's method (7), was not released by hydrolysis of the barium salt for seven minutes in *N* hydrochloric acid at 100°C. Oxidation with 0.1 *M* sodium periodate at pH 4.7 consumed 3.0 moles of oxidant in 58 hr. However oxidation of various samples of the barium salt either with 0.1 *M* periodic acid in 0.05 *N* sulphuric acid or with an equivalent concentration of sodium periodate at pH 4.7 released from 3 to 7% formaldehyde, measured colorimetrically using chromotropic acid.

To identify D-xylose-5-phosphate on a crystalline basis, dibrucine and distrychnine salts were prepared and their constants determined. The former was prepared by adding a stoichiometric amount of brucine sulphate heptahydrate in 15 ml. of water to 100 mgm. of barium xylose-5-phosphate dissolved in water. Barium sulphate was removed by filtration and the filtrate evaporated to dryness *in vacuo* at 35°C. The crude dibrucine salt weighed 262.8 mgm. (90% yield). When recrystallized from ethanol by rapid evaporation, it formed long colorless needles of a hydrate from which water of crystallization was completely removed by heating for two hours *in vacuo* at 50°C. The degree of hydration of the dibrucine salt varied with the conditions during its formation. On standing in room air overnight the anhydrous salt took up water exactly equivalent to an octahydrate. When equilibrated over a water – sulphuric acid solution (sp. gr. 1.420, relative humidity = 35) in a closed vessel, it combined with 6.5 moles of water. Recrystallization from absolute ethanol gave a hydrate with 3.5 H_2O. Analyses were therefore performed on the anhydrous salt. (Found: P, 3.06; pentose 14.02. Calc. for $C_{51}H_{63}N_4O_{16}P$: P, 3.04; pentose 14.72.) Pentose was determined by the modified Cohn–Volkin procedure (5) after titration of the dibrucine salt in water to pH 10 with *N*/10 sodium hydroxide. The anhydrous salt had $[\alpha]_D^{20} = -37.8$ ($c = 2.02$ in chloroform) and it decomposed without melting above 150°C.

The distrychnine salt was prepared by the same procedure followed for the dibrucine salt. Recrystallized from ethanol and ether it formed a tetrahydrate on standing in room air. (Found: P, 3.07; pentose 15.15. Calc. for $C_{47}H_{56}N_4O_{12}P.4H_2O$: P, 3.20; pentose 15.46.) The pure compound had $[\alpha]_D^{20} = -33.2$ ($c = 1.02$ in water) and like the dibrucine salt it decomposed above 150°C. without melting.

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GLYCERIDE SYNTHESIS

I. SYNTHESIS OF SYMMETRICAL DIGLYCERIDES FROM DIHYDROXY ACETONE AND ALLYL ALCOHOL[1]

By P. J. Barry[2] and B. M. Craig

ABSTRACT

1,3-Distearoxy acetone and 1,3-dipalmitoxy acetone were prepared by interesterification of the methyl esters of the fatty acids with 1,3-dipropionoxy acetone diethyl mercaptal. The 1,3-diglycerides were obtained by hydrogenation of the ketone group. Allyl tetrahydropyranyl ether was oxidized to 1-tetrahydropyranyl glycerol which was acetylated and interesterified with methyl esters of fatty acids to produce 1,3-diglycerides.

Numerous references may be found in the literature for the synthesis of symmetrical and unsymmetrical diglycerides (11). The methods used involve blocking a primary hydroxyl group as an acetal or ether, i.e. trityl, reacting the glycerol derivative with 2 moles of the particular fatty acid chloride, and subsequent hydrolysis or hydrogenolysis to yield the desired diglyceride. Clocker and other workers (2, 1, 7) have synthesized simple triglycerides readily and in good yield by the interesterification of triacetin and the methyl ester of the fatty acid with sodium methoxide as a catalyst. The advantages of interesterification are (*a*) the mild conditions for the reaction and (*b*) the use of the methyl ester directly which is the usual derivative by which the fatty acids are purified through fractional distillation and fractional crystallization. A side reaction has been found in the interesterification of triacetin and methyl esters but the product has not been identified. The present work describes the preparation of symmetrical diglycerides by interesterification using dihydroxy acetone or allyl alcohol as starting materials.

Schlenk, Lamp, and DeHaas (12) have synthesized a number of fatty acid mono- and di-esters of dihydroxy acetone using glycolic acid chloride as a starting material. The diglycerides were produced from these products by hydrogenation over Raney nickel. Dihydroxy acetone (3) and mono fatty-acid derivatives of dihydroxy acetone (12) will undergo monomer–dimer interconversions.

In the present work the ketone group was protected as a mercaptal to eliminate this difficulty. The series of reactions at the top of page 717 outline the synthesis of diglycerides from dihydroxy acetone.

The yield of 1,3-dipropionoxy acetone diethyl mercaptal was much higher than the diethyl ketal. The 1,3-distearoxy acetone diethyl mercaptal was easily separated from the interesterification and the yields were almost quantitative in the remaining steps to the diglyceride. The method represents a direct preparation of 1,3-diglycerides without a β–α shift which is usually encountered due to an initial blocking of a primary hydroxyl group in the glycerol. Dipalmitin was also prepared in the same manner. The preparation

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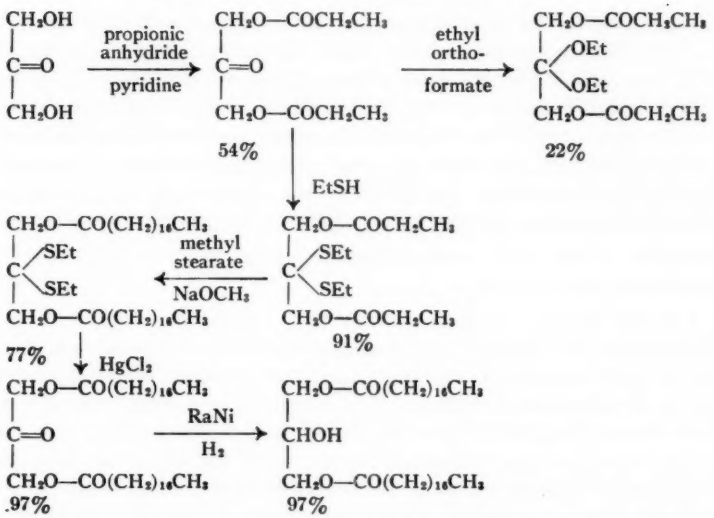
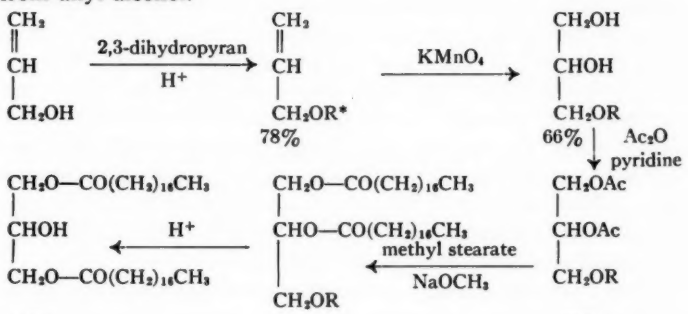
[2]*National Research Council of Canada Postdoctorate Fellow 1953–54.*



of unsaturated diglycerides would require a different procedure for reduction of the ketone group to an alcohol.

The synthesis of diglycerides from allyl alcohol required the formation of an acetal of allyl alcohol. The use of 2,3-dihydropyran for the preparation of acetals of alcohols has been investigated by Paul (10), Ott *et al.* (8), and Parham and Anderson (9). The reaction proceeds smoothly and in good yield and the acetal is readily cleaved by a mineral acid. Parham and Anderson (9) found it difficult to obtain the monoacetal of ethylene glycol. Preliminary work in this laboratory showed that it was difficult to obtain a monoacetal of glycerol. However allyl alcohol readily forms an acetal with 2,3-dihydropyran and oxidation of the ethylenic bond would yield the required monoacetal of glycerol.

The following series of reactions were followed for the synthesis of diglycerides from allyl alcohol.



Dipalmitin, 64%.

R*, tetrahydropyranyl group.

The oxidation of the allyl tetrahydropyranyl ether by $KMnO_4$ in aqueous solution at 5°C. proceeded smoothly and in fair yield. Acetylation of the 1-tetrahydropyranyl glycerol gave a low yield which may have been due to the conditions of isolation of the diacetyl derivative. The interesterification with methyl stearate produced a gel which is likely responsible for the relatively low yield of distearin. This series of reactions for the synthesis of symmetrical diglycerides merits further study, since the number of steps involved is considerably reduced as compared to conventional procedures.

EXPERIMENTAL

I. *Preparation from Dihydroxy Acetone*

(*a*) *1,3-Dipropionoxy Acetone*

1,3-Dipropionoxy acetone was prepared according to the method given by Fischer and Mildbrand (3). Five grams of (0.55 mole) dihydroxy acetone* was added to a solution of 100 ml. of dry pyridine and 120 gm. (0.923 M.) propionic anhydride, then shaken for 15 min., and allowed to stand at room temperature for two days. The solution was poured into ice water and extracted with ethyl ether. The ethereal solution was washed successively with dilute hydrochloric acid, 5% sodium bicarbonate, and water and was dried over anhydrous sodium sulphate. The ether was removed and the 1,3-dipropionoxy acetone was crystallized from petroleum ether (Skellysolve "F"). Yield, 54%; m.p., 60–60.5°C. Analysis for $C_9H_{14}O_5$: C, 53.67%; H, 6.909%. Calc.: C, 53.46%; H, 6.978%.

(*b*) *1,3-Dipropionoxy Acetone Diethyl Ketal*

The procedure used by Hurd and Pollack (4) was followed for the preparation of the diethyl ketal. Four grams of dipropionoxy acetone, 3.9 gm. of ethyl orthoformate, 5.8 gm. of ethyl alcohol, and 60 mgm. of *p*-toluene sulphonic acid were refluxed for eight hours and allowed to stand for 24 hr. The solution was neutralized with sodium ethoxide and made up to 40 ml. with water. Extraction of the aqueous solution with petroleum ether (Skellysolve "F") yielded 1.2 gm. of a yellow oil (yield 22%) which was distilled *in vacuo* to give a colorless oil, n_D^{25} 1.4296.

(*c*) *1,3-Dipropionoxy Acetone Diethyl Mercaptal*

Five grams of dipropionoxy acetone was dissolved in 50 ml. ethyl mercaptan followed by the addition of 2.5 gm. anhydrous zinc chloride. The mixture stood in a salt–ice bath for 24 hr. and was poured into a saturated aqueous solution of sodium bicarbonate. The aqueous solution was filtered and extracted with ethyl ether. The ethereal solution was dried and distilled. A colorless liquid, b.p. 157–158 at 6 mm., was obtained in 91% yield. The product was purified by distillation to yield 5.7 gm. of 1,3-dipropionoxy acetone diethyl mercaptal, n_D^{25} 1.4966. Analysis for S: found, 20.58%; calc., 20.75%.

(*d*) *1,3-Distearoxy Acetone Diethyl Mercaptal*

Three grams of dipropionoxy acetone diethyl mercaptal, 6 gm. of methyl stearate, and 0.3 ml. of a saturated solution of $NaOCH_3$ in methanol were

Supplied by Dr. A. C. Neish, Prairie Regional Laboratory, National Research Council, Saskatoon, Sask.

placed in a flask and heated on a rotary evaporator in a steam bath under vacuum. The mixture was extracted with ethyl ether and crystallized to yield 5.6 gm. (77%) of 1,3-distearoxy acetone diethyl mercaptal, m.p. 49.1–49.4°C. Analysis for S: found, 8.74%; calc., 8.79%.

(*e*) *1,3-Dipalmitoxy Acetone Diethyl Mercaptal*

Two grams of dipropionoxy acetone diethyl mercaptal, 3.55 gm. of methyl palmitate, and 0.2 ml. of a saturated $NaOCH_3$ in methanol solution were treated as in (*d*). The 1,3-dipalmitoxy acetone diethyl mercaptal was crystallized from acetone. Yield, 3.53 gm. (81%); m.p., 39.5–40.0°C. Analysis for S: 9.48%; calc., 9.52%.

(*f*) *1,3-Distearoxy Acetone*

One gram of distearoxy acetone diethyl mercaptal was dissolved in 9.5 ml. of hot acetone and 0.5 ml. of water was added. Then 2.5 gm. of $HgCl_2$ in 10 ml. of acetone was added and the mixture was refluxed for four hours. A further 25 ml. of acetone was added and the mixture was refluxed for 20 min. and filtered while hot. The residue was washed carefully with hot acetone and the 1,3-distearoxy acetone was crystallized from the acetone. Yield, 97%; m.p., 87–87.5°C.

(*g*) 1,3-Dipalmitoxy acetone was prepared in the same manner in a 97% yield with a m.p. 82–82.5°C. (reported m.p. 77–78°C. (12)).

(*h*) *1,3-Distearin and 1,3-Dipalmitin*

1,3-Distearoxy acetone, 0.67 gm., was dissolved in 75 ml. of tetrahydrofuran and was hydrogenated over Raney nickel at 50 p.s.i. for three hours at room temperature. The 1,3-distearin was recrystallized from acetone. Yield, 0.65 gm.; m.p., 78.5–79°C. (reported m.p. 79.5°C. (6)).

1,3-Dipalmitin was prepared in the same manner in a similar yield, m.p. 72.0°C. (reported m.p. 72.5 (6)). Analysis for $C_{35}H_{68}O_5$: C, 74.07%; H, 12.17%. Calc.: C, 73.89%; H, 12.04%.

II. *Preparation from Allyl Alcohol*

(*a*) *Allyl Tetrahydropyranyl Ether*

Allyl alcohol, 11.5 gm., 2,3-dihydropyran, 17 gm., and one drop of concentrated HCl were mixed at room temperature. The reaction mixture was shaken in a wrist action shaker for three hours at room temperature and was then neutralized with $NaHCO_3$. The product was distilled over $NaHCO_3$ at b.p. 165–167°C. in 78% yield, n_D^{25} 1.4421.

(*b*) *1-Tetrahydropyranyl Glyceryl Ether*

Allyl tetrahydropyranyl ether, 106.5 gm., was suspended in 450 ml. water and cooled to 5°C. in an acetone – dry ice bath. One hundred and twenty grams of $KMnO_4$ in 2550 ml. of water was added with stirring at a rate that maintained the temperature of the mixture at 5°C. ±1°C. After the addition was completed the mixture was allowed to stand in the cooling bath for two hours and was then heated for one hour on a steam bath. The solution was filtered, cooled to room temperature, and saturated with K_2CO_3. The oil was separated from the aqueous mixture, dried, and dissolved in ethyl ether.

The ethereal solution was dried over K_2CO_3 and the ether was removed to yield 102 gm. (66.5% yield) of crude product. The product distilled at 146–149°C. at 4 mm. pressure, n_D^{25} 1.4736. Oxidation of 1-tetrahydropyranyl glycerol ether with periodic acid (5) was quantitative showing a monoether of glycerol.

(*c*) *2,3-Diacetoxy-1-tetrahydropyranyl Glycerol*

Twenty five grams of tetrahydroglyceryl ether was added to a solution of 150 ml. of pyridine and 80 ml. acetic anhydride and shaken for one hour at room temperature. The mixture was allowed to stand at room temperature for two days, poured into ice water, and allowed to stand for two hours. The aqueous solution was extracted twice with ethyl ether and the ethereal solution was washed successively with water, 10% HCl, water, 5% $NaHCO_3$ solution, and water. After the solution was dried over anhydrous Na_2SO_4, the ether was removed and the product was distilled. The 2,3-diacetoxy-1-tetrahydropyranyl ether was obtained in 50% yield, n_D^{25} 1.4456, saponification value 426.5, calculated 430.7.

(*d*) *1,3-Distearin by Acyl Chlorides*

Tetrahydropyranyl glyceryl ether, 4.6 gm., was dissolved in 10 ml. of dry pyridine and 25 ml. of dry $CHCl_3$ and 10 gm. of stearyl chloride (2) dissolved in 10 ml. of dry $CHCl_3$ was added slowly to the reaction vessel. The mixture was allowed to stand at room temperature for one hour and was then refluxed for 12 hr. The product was extracted with petroleum ether (Skellysolve "F") which was washed with water, dilute hydrochloric acid, and water and was dried over anhydrous sodium sulphate. Dry HCl gas was bubbled into the petroleum ether solution for one-half hour. The solution was allowed to stand at room temperature for one hour and filtered. The 1,3-distearin was recrystallized from acetone, yield 12.6 gm. (72% of theoretical), with a m.p. 78.5–79°C. (reported m.p. 79.5 (3)). Analysis for $C_{39}H_{76}O_5$: C, 73.56%; H, 12.06%. Calc.: C, 73.55%; H, 12.10%.

(*e*) *1,3-Distearin by Interesterification*

2,3-Diacetoxy-1-tetrahydropyranyl glyceryl ether, 2.6 gm., was reacted with 6.0 gm. methyl stearate and 0.2 ml. of saturated solution of $NaOCH_3$ in methanol under vacuum at 90–95°C. Petroleum ether (Skellysolve "F") was added to the mixture and the soaps were removed by filtration. The solution was dried over anhydrous Na_2SO_4 and the salt was then removed by filtration. Dry HCl gas was passed into the solution for one-half hour and allowed to stand for one hour at room temperature. The precipitate was filtered and crystallized from acetone to yield 3.95 gm. (64% yield) of 1,3-distearin, m.p. 78–79°C. (reported 79.5°C.). Analysis for $C_{39}H_{76}O_5$: C, 73.41%; H, 12.07%. Calc. for C, 73.55%; H, 12.10%.

(*f*) 1,3-Dipalmitin, m.p. 72.5–73.5°C. (reported m.p. 72.5°C. (6)), was prepared in a similar manner in 63% yield.

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NOTES

TETRAETHYL PYROPHOSPHATE LABELED WITH PHOSPHORUS-32

By J. R. Robinson

A supply of P^{32}-labeled tetraethyl pyrophosphate (TEPP) was required for research at this laboratory and, as no details of its synthesis[1] were found in the literature, the method described here was developed. The specific activity of the product was low, 65 microcuries per gram, but this could be increased by altering the ratio of active to inactive phosphorus and no major modification of the technique would be required.

EXPERIMENTAL

Labeled phosphoric acid,[2] suitably diluted with inactive orthophosphoric acid, was converted *via* silver phosphate to triethyl phosphate using reactions reported by Baldwin and Higgins (1).

$$PO_4^{\equiv} + 3Ag^+ \xrightarrow{\text{pH 7}} Ag_3PO_4$$

$$Ag_3PO_4 + 3C_2H_5Br \xrightarrow{\text{Heat}} (C_2H_5)_3PO_4 + 3AgBr$$

The final step of the synthesis utilized a reaction reported by Bell (2).

$$2(C_2H_5)_3PO_4 + SOCl_2 \xrightarrow{\text{Heat}} (C_2H_5)_4P_2O_7 + 2C_2H_5Cl + SO_2$$

Since the experimental technique is novel the details are reported in full.

Tetraethyl Pyrophosphate

Labeled triethyl phosphate (13.29 gm., 72.4 millimoles, 47 μc. per gm.) was placed in a Hickman vacuum still[3] and treated with 4 ml. (6.6 gm., 55.6 millimoles) of thionyl chloride. The still was fitted with a receiver and a calcium chloride tube. The system was suspended about one-half inch above a small hot plate and a thermometer was fixed in a horizontal position with its bulb touching the center point of the bottom of the still. Heating was adjusted until the thermometer was steady at 70–75° and this temperature was maintained for 24 hr. The temperature was then raised to 140° for half an hour to ensure completion of the reaction. During the total reaction time 1.5 ml. of excess thionyl chloride collected in the receiver while the sulphur dioxide and ethyl chloride passed off through the calcium chloride tube. The still was allowed to cool and then was fitted to a vacuum manifold. With the pressure at 10 mm. and the thermometer indicating 120° the last traces of reactants

[1] *The use of radioactive TEPP has been reported (3, 4, 5, 6, 7). References (3) and (4) were concerned with labeled hexaethyl tetraphosphate (HETP) which probably contained TEPP as the active principle.*

[2] *One millicurie of P^{32} as phosphoric acid, obtained from Atomic Energy of Canada Limited, Chalk River, Ontario.*

[3] *Fisher Scientific Co., Cat. No. 9–124, modified by the addition of two ground-glass joints.*

and by-products were removed. After one-half hour the pressure was lowered to 0.002 mm. and the tetraethyl pyrophosphate was distilled into weighed receivers. A forerun of 1.5 ml. (optimum quantity determined by inactive runs) was discarded and 6.4 gm. (61%) of TEPP was collected, b.p. 95–100°/ 0.002 mm., n_D^{25} 1.4174. (The boiling point and refractive index were determined on inactive samples produced in the same manner.) The corrected specific activity was 65 μc. per gm., representing a recovery of 41% of the initial radioactivity. The over-all chemical yield, also based on phosphoric acid, was 49%.

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IDENTIFICATION OF DIHYDROCONIFERYL ALCOHOL

By E. A. Kvasnicka, R. R. McLaughlin, and S. Reid

Dihydroconiferyl alcohol (3-(3-methoxy-4-hydroxyphenyl)-1-propanol) (7) is an important degradation product of lignin. It has been found not only in hydrogenolysis reaction mixtures (3, 9) but also after degrading naturally occurring related products (5).

Recently, this compound was found to be present in spruce sulphite liquor (6). At the same time, dihydroconiferyl alcohol was also found in the oxidized sulphite liquors (8).

During these investigations, extensive use has been made of the available methods of identification which are described in these papers. However, an important fact is reported here because of its special interest for those working at present in this field.

The UV spectra of dihydroconiferyl alcohol and similar compounds were reported by Schuerch (9), who found an absorption maximum of 265 mμ (determined in butanol). This value appeared to be in disagreement with the known rule that hydroxy groups in the γ-position in the side chain cause only negligible shifts of the UV absorption curve of the parent hydrocarbon (2). The UV absorption maximum of this hydrocarbon, coerulignol, is 281 mμ (1, 4).

In view of this discrepancy, we re-examined the spectrum of dihydroconiferyl alcohol. A sample was synthesized according to the procedure of Schuerch (9) and characterized by the refractive index and the preparation of the known derivatives (5).

We found absorption maxima of 281.6 mμ (solvent: 95% ethanol) and 281.8 mμ (solvent: *n*-butanol).

Therefore, dihydroconiferyl alcohol cannot be regarded as an exception to the above-mentioned rule.

EXPERIMENTAL PART

The sample of dihydroconiferyl alcohol was prepared by reduction of ethyl hydroferulate with lithium aluminum hydride according to the literature (9). The compound was characterized by the preparation of the benzoate and *p*-nitrobenzoate and the purity was checked by the refractive index and paper chromatography (6).

The spectra were determined with a Beckman model DU spectrophotometer, and the resulting data appear in Table I.

TABLE I

ULTRAVIOLET SPECTRUM OF DIHYDROCONIFERYL ALCOHOL

Solvent	Concentration, mgm./l.	Maximum, mμ	Molar extinction
95% ethanol	27	281.6	2871
95% ethanol + 0.5 mole KOH/l.	27	297	3845
n-butanol	30	281.8	
n-butanol + 0.5 mole KOH/l.	30	297	

NOTE: *Equal extinction values for both neutral and alkaline solutions: 268.6 mμ.*

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